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CONTENTS.

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EFFECTS OF VENOUS HÆMORRHAGE AND INTRAVENOUS INFUSION IN DOGS.[1]

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CONTENTS.

[1] A summary of some of the results obtained was presented at the meeting of the American Physiological Society in December, 1899, and published in the Proceedings of the Society (This journal, 1900, iii, p. xxviii).

METHOD OF INVESTIGATION.

A SERIES of dogs were bled and immediately infused with various saline solutions. During these operations records of the pulse and respiration were kept, from which it was hoped to determine the relative values of these various saline solutions. Numerous blood examinations were also made for the purpose of studying the process of regeneration of the blood. The following is a more detailed account of the methods employed.

Anæsthesia. — One hour before the operation 0.05 to 0.10 gram of morphia was given hypodermically. During the operation complete anæsthesia was maintained with ether. When, however, the œsophageal balloon had been inserted chloroform was necessarily substituted for ether.

Bleeding. — Two cannulas were inserted into the jugular vein, one peripheral, the other central. From the former an amount of blood varying from 1.7 to 5.5 per cent of the body weight was allowed to flow; into the latter, various saline solutions were infused in amounts approximately equal to that of the blood withdrawn. The amount of hæmorrhage, its duration and other data are given in the accompanying table (pages 4 and 5). Strict antiseptic precautions were observed and in no case was there any subsequent suppuration, a fact of importance in relation to the leucocytes.

Infusion. — The compositions of the fluids infused were as follows : —

1.	Normal saline . .	No. 1. 0.8% NaCl.	No. 2. 1.0% NaCl.
2.	Ringer's solution .	No. 1. 0.8% NaCl.	No. 2.[1] 0.8% NaCl.
		0.026% $CaCl_2$.	0.01% $CaCl_2$.
		0.03% KCl.	0.01% $NaHCO_3$.
			0.0075 KCl.
3.	Milk	0.8% NaCl ten parts.	
		Milk one part.	
4.	Alkaline solution	0.8% NaCl.	
		0.5% $NaHCO_3$.	

The water used in making these solutions had been distilled in glass vessels. The salts had been purified by repeated recrystallization. All the solutions, except the milk, entered the circulation in a perfectly sterile condition.

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[1] This is the solution employed by Paul Maass in his recent work on the isolated mammalian heart. Archiv f. d. ges. Physiol., Bonn, 1899, lxxiv. p. 281.

The infusion apparatus consisted of a Mariotte bottle immersed in water which was kept at a constant temperature. By means of a siphon bottle air could be drawn through the fluid in the Mariotte bottle, which had been de-aërated by sterilization. The temperature of the fluid infused varied from 39° to 43° C., the average being about 41° C. These were the temperatures recorded by a thermometer in the Mariotte bottle, and there was a fall in the temperature of the fluid amounting to about 1° C. during its transit from the bottle to the vein.

Recording apparatus. — Records of the pulse and respiration were obtained by the following device.[1] A small toy balloon was fastened to the end of a glass tube so as to cover a terminal and several lateral openings. This tube was then passed down the œsophagus until the balloon was at about the level of the base of the heart, and a position was found at which an optimum record could be obtained. The external end of the tube was connected by means of a lead pipe with a sensitive tambour, the lever of which wrote on smoked paper. Experiments with the water manometer and the Hürthle manometer showed the above device to be the most satisfactory. For a recording surface smoked paper was used, each sheet measuring 2500 × 25 cm., which was sufficient for a record of 20 to 30 minutes' duration. For carrying this paper a Hurthle kymographion was employed.

This method was not perfectly satisfactory as far as the pulse was concerned. Occasionally the heart-beats became so feeble that they were not recorded, or the respiration became so violent or irregular that the record of the heart-beats was obscured. But control experiments previously undertaken on cats and dogs for the purpose of testing the efficiency of this method showed that the tracings could be relied upon to indicate any variation in the rate and character of the respiration, and that, in general, the rate and relative force of the heart-beats were indicated with a fair degree of accuracy at a time when they were too feeble to be recorded by a mercury manometer connected with the carotid.

Blood examinations. — The blood required for examination was obtained by cutting with a sharp scalpel a small slit in the margin of

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[1] A method similar to this was employed by Léon Fredericq who, however, inserted the œsophageal sound through a lateral opening in the œsophagus De l'action physiologique des soustractions sanguines, Mémoires Académie royale de Médecine de Belgique, Bruxelles. 1886, viii, pp. 1–103.

the ear. For the purpose of estimating the number of corpuscles per cubic millimetre the Thoma-Zeiss hæmocytometer was used and the amount of hæmoglobin was estimated by means of von Fleischl's hæmoglobinometer.

For the study of the erythroblasts and leucocytes cover-slip smears were obtained. These were fixed either by heating on a copper triangle for two hours at 120° C. or by keeping them twenty-four hours in a mixture of equal parts of absolute alcohol and ether. The stains employed were Ehrlich's triacid stain, Ehrlich's hæmatoxylin-eosin, Loeffler's methylene-blue, polychrome-methylene-blue

TABLE I.

Name of dog and of fluid infused	S'_1	S'_2	S'_3	S'_4	S'_5
Weight of dog in kilos	5	8.2	7.6	8.1	20
Amount of hæmorrhage in percent of body weight	2.9	2.7	5.3	4.3	4.5
Duration of hæmorrhage in minutes	10.6	2.2	2.3	2.3	28.8
Ratio of amount infused to amount of hæmorrhage	75	110	140	100	125
Temperature of fluid infused	41	41	39	41	41
Duration of infusion in minutes	14	9	4.5	8.8	3.7
Hæmorrhagic fall. Erythrocytes	65	83	94	67	61
" " Hæmoglobin	94	93	88	69	45
" " Leucocytes	77	81	78	20	44
Result of operation	R	R	R	R	D
Posthæmorrhagic fall. Erythrocytes	65	75	80	42	—
" " Hæmoglobin	73	68	56	23	—
Posthæmorrhagic leucocytosis	225	100	134	123	—
Duration of posthæmorrhagic fall in days. Erythrocytes	5	3	8	4	—
" " " " Hæmoglobin	3	3	12	3	—
Day of recovery of erythrocytes	7+	5+	24	25+	—
" " " " hæmoglobin	7+	5+	32+	25+	—
Duration of observations in days	7	7	32	25	—

and dahlia. The lenses used were $\frac{1}{12}$ Leitz oil-immersion and a No. 4 Zeiss ocular. The measurements were made with the aid of a camera lucida and a stage micrometer. In determining the

relative numbers of the various forms of leucocytes one thousand individuals were usually counted.

The numbers expressing the hæmorrhagic and posthæmorrhagic fall represent in percentages the ratio of the number of corpuscles or the amount of hæmoglobin after hæmorrhage to the number or amount before hæmorrhage.

The date at which the number of erythrocytes and hæmoglobin became normal is often unknown owing to the discontinuance of the observations. Such figures as 7+ signify that at the end of seven days the count had not returned to normal.

TABLE I *(continued)*.

R'	R'₂	R'₃	R'₄	R'₅	R'₆	M	R''₁	R''₂	S'₁	Sᵃ	S'₆	S'₇
7	9.4	12	9	6	9.9	7.4	10	13	7	12	18	30
1.7	2.6	3.2	2.7	4.6	4.0	5.5	5.2	5.4	4.2	3.8	4.4	4.3
6	5	3.3	5.1	3	5	7.1	—	—	6.6	30	—	—
100	100	100	166	140	133	100	100	100	100	92	100	100
41	39	41	43	42	42	41	41	41	43	41	40	41
—	6	5.6	6.8	5.5	6	4.2	—	—	4.8	10	—	—
58	—	94	—	—	—	63	70	62	75	94	—	—
—	—	—	—	—	—	62	74	59	78	82	—	—
—	—	50	—	—	—	23	43	41	—	44	—	—
R	D	R	D	D	D	R	R	R	R	R	R	R
—	—	69	—	—	—	43	38	48	58	65	66	57
—	—	—	—	—	—	37	27	37	50	33	41	45
—	—	222	—	—	—	257	165	91	—	358	—	—
—	—	5	—	—	—	4	5	7	2	5	—	4
—	—	—	—	—	—	3	5	3	5	2	—	4
—	—	15	—	—	—	12+	11+	11+	5+	5+	4+	15+
—	—	—	—	—	—	12+	11+	11+	5+	5+	4+	15+
1	1	15	1	1	1	12	11	11	5	5	4	15

ABBREVIATIONS. — S', normal saline solution No. 1; S'', normal saline solution No. 2; Sᵃ, alkaline solution; R¹, Ringer's solution No 1; R², Ringer's solution No. 2; M, milk (see page 2); R, recovery; D, death.

THE IMMEDIATE EFFECTS ON THE PULSE AND RESPIRATION.

As stated above, the cardiac and respiratory movements were recorded by means of an œsophageal balloon connected with a tambour. Tracings were obtained on a Hürthle kymographion from 15 dogs. With the data thus obtained pulse and respiration charts were constructed so that the variations in the frequency of the heart-beat and in the depth and rate of the respiratory movements could be seen at a glance, which greatly facilitated comparison.

The amount of blood withdrawn varied from 1.7 to 5.5 per cent of the body weight, the usual quantity being about 4.5 per cent. The solutions infused and other details are given in the accompanying table (pages 4 and 5).

Hæmorrhage. — The invariable effect of hæmorrhage was to weaken the heart-beats sometimes to such an extent that the tambour ceased to record them. There was also an acceleration[1] which varied in a general way with the severity of the hæmorrhage. This acceleration might come on quickly either at the beginning or near the end of the hæmorrhage, or there might be a constant gradual increase in the heart rate throughout the hæmorrhage. In other cases the acceleration was preceded by a brief period of slowing. The acceleration usually persisted to the cessation of the hæmorrhage, but was sometimes succeeded by a short period in which the rate was slightly slowed.[2]

Infusion: — a. *Recoveries.* — The effect of infusion on the rate and strength of the heart-beat and on the depth and frequency of the respirations was not striking. There was of course a gradual return to normal which sometimes did not appear until after the infusion had been discontinued. That the improvement was due to the infusion would seem doubtful when the amounts of blood withdrawn were as small as 1.7 to or 2.7 per cent of the body weight, but since most of the dogs lost from 4 per cent to 5.5 per cent of the body weight the beneficial effect of the infusion is obvious.[3]

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[1] According to Fredericq (*loc. cit.*) acceleration occurs only in those animals in which the vagus is in tonic action.

[2] According to Fredericq (*loc. cit.*) this slowing is due to vagus excitation.

[3] Fredericq's figures (*loc. cit.*) are as follows: —

> 0 to 2.3 % body weight, loss readily borne;
> 2.3 to 4.5 or 5 % body weight, dangerous;
> over 4.5 or 5 % body weight, fatal.

The experiments of Oswald Feis have led this author to doubt the efficacy of

The interesting and important result is this, that as far as the pulse and respiration are concerned all the fluids used (except Ringer No 1.) acted equally well. Moreover the rapidity and character of the regeneration of the blood appeared to be uninfluenced by the composition of the fluid infused.

b. *Fatalities.* — The fatal cases were five in number, four being during infusion with Ringer No. 1, one being after infusion with 0.8 per cent NaCl. Of the first group, the " Ringer group," all four died shortly after the infusion had been begun. Two of these, R'$_4$ and R'$_5$, had before death marked spasms with violent respirations. The tracing from another, R'$_2$, corresponded exactly to that obtained by Bergendal[1] in cases of death by hæmorrhage, while in the fourth, R'$_6$, the respiration and pulse became gradually weaker until at last they ceased entirely.

The death of the dog S'$_3$, infused with 0.8 per cent NaCl, differed very much from the deaths in the Ringer group. It occurred two hours after the infusion had been discontinued. The blood count made one hour before death showed a somewhat greater diminution in the number of erythrocytes than might have been expected and the anæmia found at autopsy was extraordinary. It is suggested that the total amount of blood present in the animal may have been less than its body weight would lead one to suspect, and that the infusion was responsible for its surviving the hæmorrhage two hours rather than for its ultimate death.

The fact that all of the fatalities, except one, occurred in dogs which were being infused with Ringer No. 1 points to the composition of the fluid as the cause of death, especially when we consider that in two of the cases the loss of blood was comparatively slight. It is possible that the high percentage of calcium in Ringer No. 1 may have overstimulated the heart. It is interesting to note that although the hæmorrhages were on the whole more severe, recovery took place in the two dogs infused with Ringer No. 2 in which the percentage of calcium was considerably lower.

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sodium chloride infusions: Archiv für pathologische Anatomie [etc.], Berlin, 1894, cxxxviii, p. 75.

[1] K. Bergendal: Ueber die bei der acuten Verblutung an dem Kreislauf- und Athmungsapparaten auftretenden Erscheinungen, Skandinavisches Archiv für Physiologie, 1897, vii, p. 186.

THE REGENERATION OF THE ERYTHROCYTES AND HÆMOGLOBIN.

As stated above, blood examinations were made before and after hæmorrhage and were repeated at intervals for some time. The data thus obtained were used in the preparation of the accompanying table (pages 4 and 5) and of a number of blood charts of which two examples are given (pages 9 and 10).

The erythrocytes of the dog vary in size from 6μ to 8μ, the average being 7μ. They are present in the normal blood in numbers ranging from 6,250,000 to 8,500,000 per cubic millimetre, the average being 7,215,000 (15 dogs examined). The hæmoglobin varies from 59 per cent to 98 per cent, the average being 75 per cent (12 dogs examined).

Description of blood charts. — The accompanying blood charts (Fig. 1, etc.) show certain important facts which are, however, already well known. They show a fall in the number of the erythrocytes and in the amount of hæmoglobin. This fall takes place in two stages. First, there is a primary (hæmorrhagic) fall which is due to the removal of the corpuscles from the circulation by the bleeding while the amount of the plasma is kept constant by the infusion and the withdrawal of the lymph from the tissues. Second, there is a posthæmorrhagic[1] or secondary fall which occurs after the bleeding has ceased and must therefore be due only indirectly to this cause. According to this definition the primary fall ceases at the moment when the bleeding ceases while the posthæmorrhagic fall beginning at the cessation of the bleeding continues for some time. This posthæmorrhagic fall of the erythrocytes continues for four to eight days, the number of corpuscles sometimes reaching a minimum of 42.5 per cent of the original number. The hæmoglobin also falls for three to twelve days and may reach a minimum of 23 per cent of the amount originally present.

A more careful examination of these and similar charts and of the table on pages 4 and 5, leads to the following conclusions: — *first*, the extent of the hæmorrhagic and the posthæmorrhagic fall of erythrocytes and of hæmoglobin is not closely dependent on the severity of the hæmorrhage; *second*, the hæmorrhagic fall of the erythrocytes is sometimes more, sometimes less than that of the hæmoglobin;

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[1] This posthæmorrhagic fall has been frequently noted clinically and also by Guy L. Kiefer, Medical News, 1892, lx, p. 225, in experimental work on dogs.

third, the posthæmorrhagic fall of the erythrocytes is usually less proportionally than that of the hæmoglobin; *fourth*, the primary fall of the erythrocytes does not vary with that of the hæmoglobin; *fifth*, the primary fall of the hæmoglobin does not always bear any relation to the posthæmorrhagic fall; *sixth*, the number of erythrocytes returns to normal sooner than the amount of hæmoglobin; *seventh*, the character and duration of the erythropenia and of the diminution
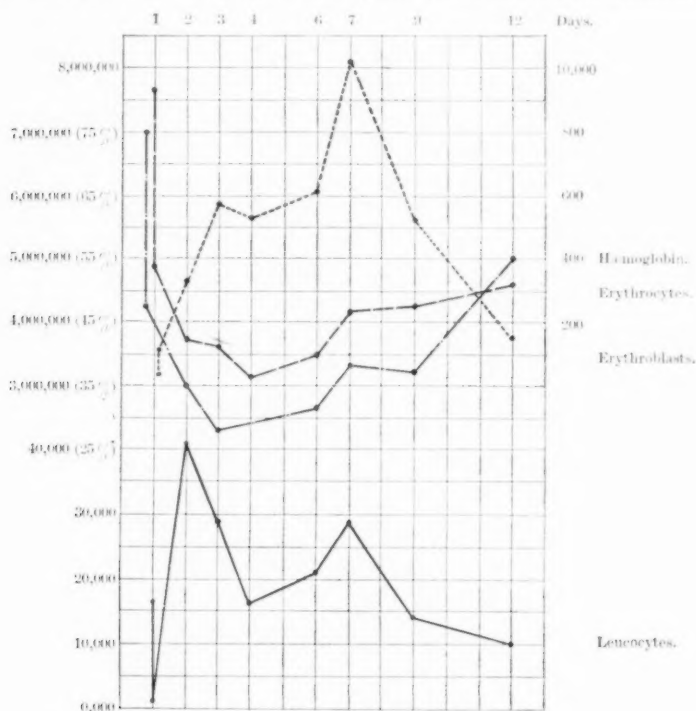
FIGURE 1. Dog M.

of the hæmoglobin are not obviously closely dependent on any known factors; *eighth*, apparently the amount, composition and temperature of the fluid infused and the rapidity of the process of infusion were without effect on the course of the regeneration of the blood.

Cause of the posthæmorrhagic fall. — As already stated, the number of the erythrocytes and the amount of the hæmoglobin continue to

Percy M. Dawson.
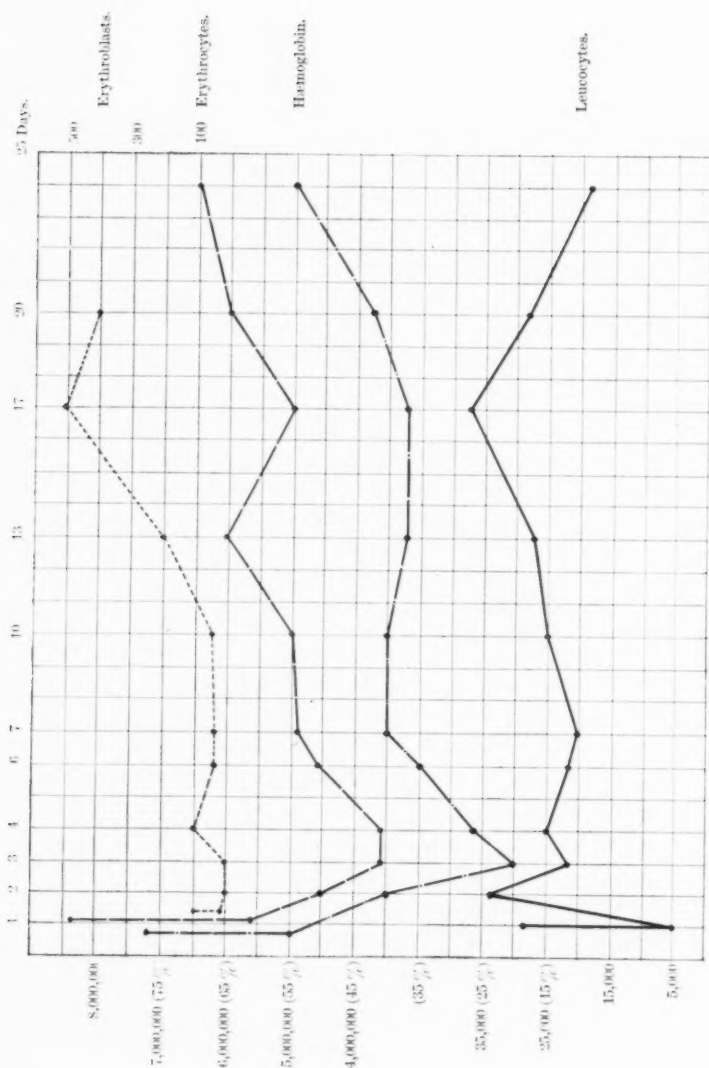
FIGURE 2. Dog S_4.

fall after the cessation of the hæmorrhage and hence the fall may be divided into a primary or hæmorrhagic fall and a secondary or posthæmorrhagic fall. The cause of the former is of course the removal by bleeding of the corpuscles from the circulation while the plasma is being constantly replenished by abstraction of lymph from the tissues, and, in the cases here reported, by the liquid infused. The cause of the latter is, however, not so obvious. This posthæmorrhagic fall is such a constant and striking phenomenon that an especial effort was made to throw some light upon its etiology.

Two factors are concerned in preserving the constant number of erythrocytes per cubic millimetre of blood. These are, first, the relation of the amount of plasma to the number of corpuscles; second, the relation of the production of erythrocytes to their destruction, that is, to the duration of the life of the individual corpuscles. Hence two corresponding explanations at once suggest themselves. *First*, it might be supposed that the gradual replenishing of the plasma after hæmorrhage might cause the posthæmorrhagic fall, just as the replenishing of the plasma during hæmorrhage causes the hæmorrhagic fall. But the replacing of the plasma is a process which does not require a period of more than a few hours to two days even in cases in which there has been no saline infusion, while the posthæmorrhagic fall of erythrocytes and hæmoglobin does not reach the maximum before the fourth to eighth and third to twelfth day respectively. Moreover, in the experiments above described the plasma was replaced immediately after the bleeding by the infusion of saline solutions. Hence this explanation may be dismissed.

Second, any shortening of the lives of the individual corpuscles would, if unaccompanied by a corresponding increase in their production, cause a decrease in the number of erythrocytes in the blood. The number would continue to fall until equilibrium had been established between production and destruction. After this, any increase in the production of corpuscles or the lengthening of the life of the corpuscles or both factors acting together, would cause a gradual return to normal. Such a shortening of the life of the corpuscles must be attributed either to changes in the corpuscles themselves or to changes in their environment, the plasma.

a. Changes in the plasma. — The most obvious possible change in the plasma which could cause an early disintegration of the corpuscles would be a variation in the osmotic pressure of the plasma.

The presence or absence of such a change could be shown by comparing the serum of the same dog before and after bleeding.

A large dog, S'₆, having a blood count of 6,232,000 erythrocytes and 66 per cent hæmoglobin was freely bled and the freezing point of the serum determined by means of Beckman's apparatus. On the fourth day, when the blood count had fallen to 2,640,000 erythrocytes and 27 per cent hæmoglobin, the freezing point of the serum was again determined, and found to coincide exactly with that of the normal animal. Hence the posthæmorrhagic fall is not due to changes in the osmotic pressure of the plasma.

b. Changes in the corpuscles. — In order to determine the relative osmotic tensions of the corpuscles before and after hæmorrhage the following method was adopted. Solutions of sodium chloride of varying concentration (0.6 to 0.9 per cent) were prepared. About 0.1 c.mm. of blood, obtained in the usual manner from the ear of the dog, was added to 1 c.cm. of each of these solutions, the mixing being done in the pipette of the Thoma-Zeiss hæmocytometer. Each specimen was then blown into a small glass-stoppered vial. Every four hours for twenty-four hours, and also at the end of thirty-six hours, the colors of the solutions were noted and the sediment consisting of corpuscles was examined with the microscope. The course of events in each of these vials was essentially the same, differing only in time relations. For the sake of convenience the process of laking and disintegration may be divided into three stages. First, the corpuscles settle, forming a distinct red sediment under a clear transparent fluid. They are variously distorted, but no efforts were made to discover the significance of these variations. Second, the fluid gradually becomes tinged with hæmoglobin, while the sediment and the corpuscles composing it are seen to become correspondingly paler. Third, along with the loss of color the sediment is gradually dissolved and the corpuscles after becoming ghosts disappear entirely.

The accompanying table (page 14) shows the results of one of three experiments conducted as above described. In this table the following abbreviations have been employed: c., fluid clear; f. t., faintly tinged; t., tinged; d., corpuscles disappeared. An examination of the table shows that the corpuscles which occur in the circulation after hæmorrhage and infusion are much less resistant than normal corpuscles, that is, the corpuscles laked more rapidly than in the normal animal in solutions either isotonic or slightly

TABLE II.

Per cent.	Time in hours.								Dog. S 7
	4	8	10	12	16	20	24	36	
0.6	t	t		t	t	d			
0.7	c	c		f.t.	t	t	t	t	Before bleeding.
0.8	c	c		f.t.	t	t	t	t	Erythrocytes 6,957,333. Hæmoglobin 87%.
0.9	c	c		f.t.	t	t	t	t	
0.6	t		d						
0.7	t		t		d				4th day.
0.8	t		t		d				Erythrocytes 4,045,333. Hæmoglobin 40%.
0.9	c		c		c	d			
0.6	f.t.	d							
0.7	f.t.	d							8th day.
0.8	c	c		t	d				Erythrocytes 4,692,666. Hæmoglobin 45%.
0.9	c	c		c	t		d		
0.6	t	t		d					
0.7	t	t		t	t	t	t		15th day.
0.8	c	c		c	c	t	t		Erythrocytes 6,205,333. Hæmoglobin 60%.
0.9	c	c		c	c	c	t		

hypotonic to the serum. This would seem to indicate that probably the newly formed corpuscles are at first laked and destroyed by the animal's own serum. Moreover, it can be seen that the increase in the number of erythrocytes began before there was any appreciable increase in the resistance of the corpuscles, a fact which may be accounted for by supposing that an increased production of erythrocytes had already begun to compensate for the decreased duration of their individual existences in the circulation.

c. Conclusion. — To recapitulate, it seems probable that after hæmorrhage immature erythrocytes having an osmotic tension above normal, and hence being less resistant than the prehæmorrhagic corpuscles find their way into the circulation; that as the older prehæmorrhagic corpuscles are replaced by the immature posthæmorrhagic individuals a constant decrease owing to laking occurs in the number of erythrocytes; that this decrease continues until equilibrium is reached between production and destruction; that the subsequent rise is due at first to an increase in the rate of production and then to the fact that more mature and hence more resistant corpuscles pass into the circulation.

The method employed was too crude to determine the time at which the increased production began or whether it was preceded by a period of diminished production, but merely indicated the point of time at which the process of destruction ceased to predominate over that of production.

THE ERYTHROBLASTS.

Various forms of erythroblasts occur in the blood of dogs, both under normal conditions and especially after hæmorrhage. In the normal blood (eight dogs examined) they are present in numbers ranging from somewhat less than 22 to as many as 560 per c.mm. of blood, while the maximum noted after hæmorrhage was 1050 per c.mm. Although there are variations in the size of the cells and in the avidity with which the nucleus takes up stains, none of the forms differs markedly from the normoblast type. The most abundant form has the size of an erythrocyte and a nucleus which stains intensely black with triacid. Other forms differ from these either in being larger (the largest being about the size of a polymorphonuclear leucocyte, 12μ), or in having a somewhat paler nucleus, or in

both these particulars. As a rule, the larger the erythroblast the paler the nucleus, but this is by no means invariably the case.

On examining the blood charts on which the variations in the number of erythroblasts after hæmorrhage with infusion are recorded (Figs. 3, 4, 5, 6,) the following course of events may be noted. There is a posthæmorrhagic increase which may be preceded by a temporary decrease. This temporary decrease does not depend upon the severity of the hæmorrhage nor on the fluid infused nor does it bear any apparent relation to the amount or duration of the posthæmorrhagic fall of the erythrocytes. The posthæmorrhagic increase reaches its maximum in from one to seven days. This maximum may be followed by a decrease and then by a second increase.

The preceding statements are based on observations made on four dogs.

THE LEUCOCYTES.

The number of leucocytes in the normal dog, determinations being made at least 12 hours after meals, ranges from 11,000 to 28,000, the average being 19,300 (12 dogs examined).

Morphology. — As the morphology of the leucocytes has not been extensively studied in the dog it has seemed advisable to present a somewhat detailed description of the different varieties.[1]

a. Polymorphonuclear leucocytes. — The polymorphous nucleus has usually a sharply defined outline. With the triacid stain it is purplish or bluish, and the chromatin network with its netknots is usually quite distinct. In the contiguous cytoplasm one usually sees four to ten perinuclear granules, which stain intensely black. They are usually small, but may at times be almost as large as a lobe of the nucleus and are then much less deeply stained.

The cytoplasm and the neutrophilic granules should be considered together. The latter may be large, clearly defined, and purplish in color, and in this case the cytoplasm is often clear and unstained, or

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[1] Hans Hirschfeld has given very brief descriptions of the leucocytes occurring in a number of the mammalia: Arch. f. path. Anat. [etc.], Berlin, 1897, cxlix. p. 22. More extended descriptions are given by T. W. Tallqvist and E. A. von Willebrand in the Skandinavisches Archiv für Physiologie, 1899, ix. p. 37. The work of these authors appeared in December, 1899, at a time when the researches described in this article were practically completed.
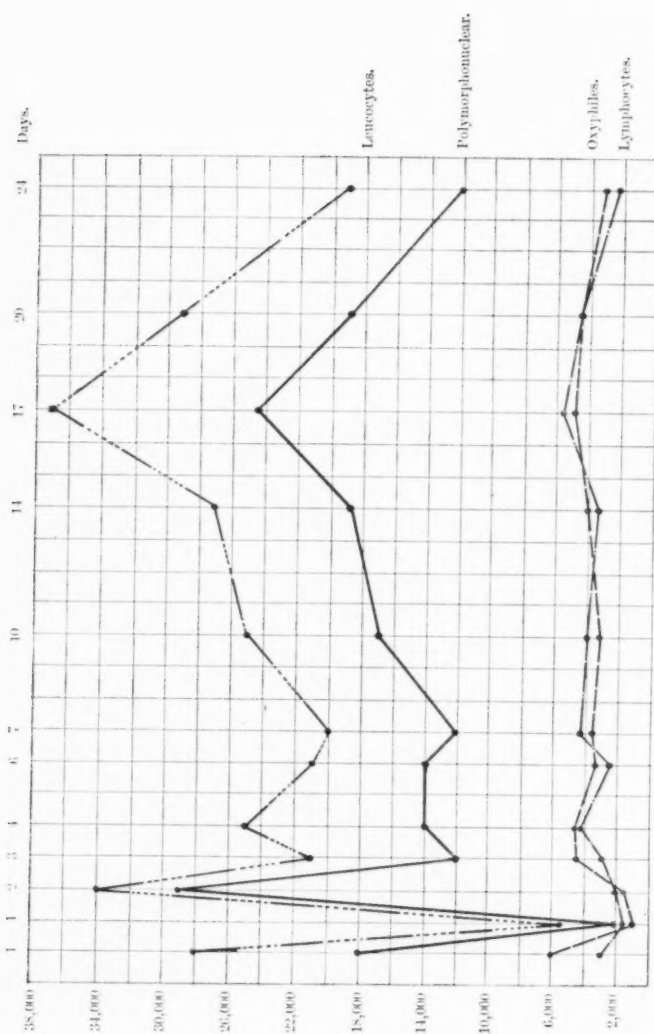
FIGURE 3. Dog S'4.

the granules may be smaller, sometimes so small as to be at the limit of vision, or there may be no granules visible. In proportion to their smallness the outlines of the granules become more indistinct and the color of the cytoplasm approaches that of the granules until in those cells in which no granules are seen the cytoplasm is of a purplish color and the whole cell body is apparently homogeneous, as if the substance of the granules had been dissolved but without losing its staining affinities. Hence we may regard these leucocytes as being of two types, the granular and the non-granular, between which there is no sharp line of demarcation owing to the numerous intermediate forms. The non-granular variety is often small, sometimes not larger than a lymphocyte, 7μ; with a typical nucleus but dark purplish homogeneous cytoplasm. The granular variety is always large, $11\,\mu$; and the colorless cytoplasm contains distinct neutrophilic granules.

A small number of cells may be regarded as a third type. In these the perinuclear granules are absent and the nucleus has few lobes, is coarsely rounded in outline and not sharply defined (except with polychrome-methylene-blue). In other respects they resemble the second or granular type.

With polychrome-methylene-blue only the cells of the third type show cytoplasmic granulation, and the mottled appearance of the nucleus in this type recalls the nuclei of the transitional and large mononuclear varieties.

b. Oxyphiles.[1] — These leucocytes vary considerably in size, from 8 to 13 μ. The polymorphous nucleus resembles the nucleus of the third type of the foregoing variety but has often a decidedly greenish tint with triacid stain. It may be large and occupy most of the cell. The chromatin is collected into lumps and granules sharply outlined against the remaining achromatic hyaline part. In small individuals the nucleus is often deeply stained and the cytoplasm very scanty. Between the lobes of the nucleus and about the periphery of the cell are numerous oxyphilic granules. With the triacid stain they are dark red, almost port wine color. They are often very abundant and closely crowded together. When small and numerous, they are usually approximately equal in size and globular; when of medium

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[1] From the work of Hirschfeld, and of Tallqvist and Willebrand, the term oxyphile would seem to be more appropriate than that of eosinophile, and hence the former has been employed.

size they have often the form of short rods; or the oxyphilic substance may occur in irregular masses of variable, often of large, size, so that one of these masses may occupy one-eighth of the entire cell. It does not seem probable that the large masses of oxyphilic substance give rise by fragmentation to the smaller and more numerous granules, since the cells containing the former have feebly staining, indistinct nuclei and vacuolated cytoplasm suggesting a degenerative condition.

c. Lymphocytes. — The typical lymphocytes are round and of fairly constant size, measuring about 7 μ. The nuclei are usually round but vary considerably in shape, being also oval, reniform, sometimes pyriform, half-moon shaped, oblong, but only rarely somewhat dumbbell-shaped. Each nucleus is large in size and sharply outlined. Its margin is usually entire, being only rarely quite indented or slit. With triacid it is of a bright blue color with an indistinct chromatin network of a darker blue and with a nucleolus which stains intensely black. The cytoplasm is represented by a homogeneous rim of varying thickness usually very narrow, which with triacid stains a purplish color. Occasionally this rim of cytoplasm cannot be distinguished. In specimens stained with polychrome, the cytoplasm may often be seen to contain a number of reddish brown granules of varying size.

While carefully avoiding the statement that any genetic relationship exists between the various forms of leucocytes, it is to be noted that in the morphology of the leucocytes one of the most impressive features is the entire absence of sharp boundaries between the polymorphs and the lymphocytes. In making a differential count one is continually obliged to place certain individuals in one class or another, although from the standpoint of their morphology they clearly belong to both. Hence it seems that while no description which assumes a common origin for the several varieties of corpuscles is at present justifiable, still any description which fails to acknowledge the absence of sharp morphological boundaries is equally at fault.

d. Transitional and large mononuclear forms. — Taking the lymphocyte as a starting-point the series of morphologically transitional forms may be arbitrarily but conveniently divided as follows.

Class I. The cytoplasm is more abundant than in typical lymphocytes but still stains a deep purplish color with triacid.

Class II. The cells are considerably larger and are almost entirely

occupied by the nucleus, the cytoplasm forming only a slight rim. Stained with polychrome the nucleus is seen to be more mottled and less homogeneous in appearance than nuclei of the lymphocytes stained by the same reagent.

Class III. The nucleus is of the same size as in the preceding class, but the cytoplasm is more abundant, so that these leucocytes resemble the large mononuclears which occur in man. Stained with triacid the cytoplasm is usually of a purplish tint, but when polychrome is used the cytoplasm is seen to contain round or bacillar granules which are often large and stain deep blue. With the latter stain the nucleus is mottled and has a distinct coarse chromatin network. In certain cells the cytoplasm is unstained by either triacid or polychrome but remain clear and hyaline exactly resembling the large mononuclear leucocytes occurring in man; this form is, however, extremely rare.

Class IV. The cells are about 11 μ in size and the form of the nucleus varies, being indented, bilobed, trilobed, lunate, or sausage shaped. The nucleus is large and with polychrome the arrangement of the chromatin resembles that in the third type of polymorphs. In some cases the cytoplasm is very scanty.

Class V. The cell is small, the cytoplasm homogeneous, bluish or reddish according to the stain used. The nucleus varies in shape as in the preceding class, but is small and dark. These cells are few in number and resemble somewhat the small non-granular type of polymorphs.

Class VI. There is a certain number of cells of very large size, sometimes attaining a diameter of 18 μ. These cells can be studied best when stained with polychrome. The nucleus is large with indistinct margins and the chromatin is distributed throughout the nucleus which thus appears nearly homogeneous. The cytoplasm is somewhat bluish in color.

e. Degenerate forms. — Forms which may be regarded as degenerate occur, but are few in number. In these the nucleus is rounded, indistinct, homogeneous and apparently undergoing karyolysis. The cytoplasm is vacuolated, often markedly so. Any variety of leucocyte may show these degenerative changes.

f. Mast-cells. — The presence of cells having a basophilic granulation was demonstrated with dahlia and polychrome-methylene-blue. These cells vary in size from 6 μ to 13 μ. With polychrome, the nuclei remain unstained while the granules assume a bright carmine

color. The granules are highly refractive, vary in size, are bacillar, round, or lenticular in shape.

The cells are few in number, usually not more than 0.2 per cent. Three series of differential counts were made, corresponding to those previously made with the triacid stain, but no constant variation in the number of basophiles was observed. Their occurrence is, however, so infrequent and irregular that undoubtedly only a very considerable variation would have attracted attention.

The percentages[1] in which the different varieties occur are as follows (ten dogs counted):

	Per cent.		Per cent.
Polymorphonuclear leucocytes	62.4—68.0.	Average	64.56.
Lymphocytes	11.2—31.6.	"	22.17.
Oxyphilic leucocytes	2.6—21.6.	"	8.85.
Other forms	1.2— 9.4.	"	4.42.

Changes following hæmorrhage. — The changes in the leucocytes following hæmorrhage with infusion were studied in the following manner. First, the total number of leucocytes per cubic millimetre was estimated by means of the Thoma-Zeiss hæmocytometer. Next, from dogs S'_3, S'_4, R'_3 and M four series of differential counts were made. From these observations, the actual number of each variety per c.mm. was easily estimated and from these data blood charts were constructed.

It was found that the variations in the total number of leucocytes depended almost entirely on the number of polymorphs, so that the curve of the one was almost parallel to that of the other. In general it may be said that there is subsequent to hæmorrhage a marked temporary leucopenia followed by a considerable leucocytosis, which is also of short duration. Subsequent variations are often extensive, but inconstant, and have not been shown to have any particular significance.

On examination of the charts the following facts were also observed. First, the polymorphs always show a marked hæmorrhagic

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[1] Tallqvist and Willebrand (*loc. cit.*) made differential counts of the blood of 15 dogs. Their results are as follows. —

	Per cent.		Per cent.
Polymorph	68.4—75.6.	Average	70—80.
Lymphocytes	4.2—10.8.	"	5—10.
Oxyphiles	0.2— 6.6.	"	4— 8.
Other forms	9.6—17.4.	"	10—15.
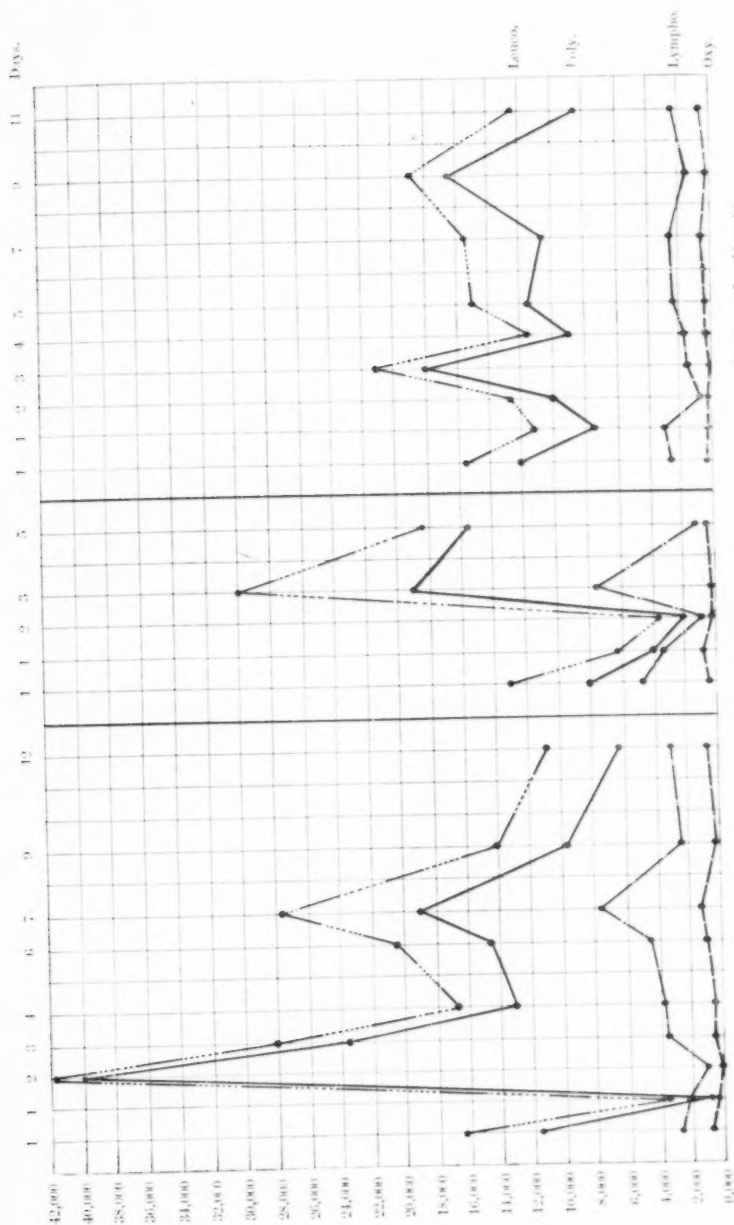
FIGURE 4. Dog M.   FIGURE 5. Dog K₂.   FIGURE 6. Dog S₂.

fall which is proportionally greater than that of the lymphocytes or oxyphiles. Second, the lymphocytes and oxyphiles usually show a hæmorrhagic fall in which either may exceed the other. Third, the polymorphs always present a posthæmorrhagic rise which usually reaches its maximum on the first day after the hæmorrhage. Fourth, the lymphocytes as a rule have a posthæmorrhagic fall commonly lasting one day, after which there is a more or less rapid rise to or above normal. Fifth, the oxyphiles usually show a posthæmorrhagic fall lasting two days, after which there is a return to normal. Sixth, the subsequent variations in the proportions of the different varieties of leucocytes and in the total number of leucocytes are irregular and are due chiefly to variations in the number of polymorphs.

Any irregularity in the behavior of any of the varieties of leucocytes is apparently without effect on the other forms. Such irregularities are the following. First, there may be a posthæmorrhagic fall in the polymorphs lasting one day and followed by the usual posthæmorrhagic rise. Second, there may be a hæmorrhagic rise in the lymphocytes and oxyphiles preceding the posthæmorrhagic fall. Third, the posthæmorrhagic rise of polymorphs may not reach a maximum until the second day. Fourth, the lymphocytes may not show any posthæmorrhagic fall.

Discussion. — Some of these facts appear to be of sufficient importance to merit special attention.

The hæmorrhagic increase in the percentage of lymphocytes ("*first*" *observation on page* 21). As there is no reason for supposing that the leucocytes are not removed by bleeding in numbers proportional to their relative numbers in the blood this marked increase in the proportion of lymphocytes after hæmorrhage remains to be accounted for. Two explanations at once suggest themselves: either an unusually rapid destruction of the polymorphs takes place, or a large number of lymphocytes is introduced into the circulation during or immediately after hæmorrhage. If the former were the case one would expect to find numerous degenerated forms present, since in some cases a destruction of 90 per cent of the polymorphs would be necessary to account for the increased percentage of lymphocytes. Whereas, as a matter of fact, only 2 per cent of the forms present could possibly be regarded as degenerate polymorphs. Moreover, this supposition would not account for those cases in which there is an actual increase in the number of

leucocytes (noted as a second irregularity on page 22). Hence we may conclude that if there is an increased destruction of polymorphs, the circulating blood contains no evidence of it, while in one case at least there was a definite increase in the number of lymphocytes in the circulation.

The origin of the polymorphonuclear leucocytes. — It is interesting to see to what conclusion the supposition that the polymorphs are derived from lymphocytes would lead one in accounting for the posthæmorrhagic increase in the former. In the case of dog M (see Fig. 4, page 21) 3000 lymphocytes normally kept up a supply of 11,000 polymorphs. After hæmorrhage there were only 2,000 lymphocytes and these would be obliged to increase and pass through their metamorphoses fast enough to form at least 31,500 polymorphs in twenty-four hours. This would require an enormous activity and one would expect to find a larger number of transitional forms than usually occur. Only 2.2 per cent of these latter were actually found, of which 1.4 per cent were possibly only degenerated polymorphs, a number slightly less than normal. This scarcity of transitional forms points to the conclusion that whatever may be the origin of the polymorphs, there is no evidence that they are derived from the lymphocytes, at least not in the circulating blood.

The fate of the polymorphonuclear leucocytes. — During the fall which succeeds the posthæmorrhagic leucocytosis the number of undoubtedly degenerated polymorphs is only slightly although distinctly increased. Hence there is no conclusive evidence that any extensive destruction of the polymorphs occurs in the circulating blood.

Behavior of the lymphocytes. — For the variations in the number of lymphocytes the following explanation is suggested, that after all the available lymphocytes have been swept into the circulation during the regeneration of the plasma, thus causing the hæmorrhagic rise or at least modifying the hæmorrhagic fall, some time is required for the compensatory proliferation to become evident and during this period the posthæmorrhagic fall is observed.

CONCLUSIONS.

The results of the investigations above described have led to the following conclusions : —

1. From observations on the pulse and respiration there is no

evidence that variations (within the limits above described) in the composition of the fluid infused have any influence on the immediate recovery of the animal.

2. Examinations of the blood afford no evidence that such variations have any influence on the character and rapidity of the regeneration of the blood.

3. Ringer's solutions containing as much as 0.026 per cent $CaCl_2$ are dangerous.

4. The posthæmorrhagic fall in the number of erythrocytes and in the amount of hæmoglobin per cubic millimetre of blood is probably due to the compensatory introduction into the circulation of immature erythrocytes, which disintegrate more rapidly than the normal erythrocytes, thus producing the posthæmorrhagic fall.

5. There is no evidence of any close relation between the number of erythroblasts and the rapidity or character of the regeneration of the erythrocytes and hæmoglobin.

6. In the hæmorrhagic leucopenia, the diminution in the number of polymorphonuclear leucocytes is proportionally much greater than that of the lymphocytes.

7. The posthæmorrhagic leucocytosis is almost entirely due to the increase in the number of polymorphonuclear leucocytes.

8. The microscopical study of the transitional and large mononuclear forms in the blood of dogs strongly favors the view that the polymorphonuclear leucocytes are derived from the lymphocytes, but the numerical relations of the several varieties of leucocytes before and after hæmorrhage afford no corroborative evidence that this is actually the case.

9. There is no morphological or numerical reason for suspecting that the oxyphiles do not form a distinct type.

ON THE ELIMINATION OF NITROGEN, SULPHATES, AND PHOSPHATES AFTER THE INGESTION OF PROTEID FOOD.

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IN interpreting the results of the experiments on metabolism which have been carried on in this laboratory and elsewhere in connection with the nutrition investigations of the U. S. Department of Agriculture, a need has been felt for more information as to the time relations of those changes in the urine which result from changes in diet or other conditions affecting proteid metabolism.[1]

An investigation of the subject has therefore been undertaken in this laboratory, and the experiments here reported represent the beginning of the inquiry. They were planned and carried out with the advice and counsel of Prof. W. O. Atwater, to whom we are also indebted for the laboratory facilities which made the work possible.

Earlier Investigations. — Naturally among the many investigators of proteid metabolism, a number have given more or less attention to its time relations. Among the earlier of these were Becher,[2] Voit,[3] Forster,[4] Panum,[5] Falck,[6] and Feder,[7] each of whom observed the course of the nitrogen excretion after a meal rich in proteids. Some of the observations were made upon dogs, some upon men. In some cases it was found that the nitrogen excretion reached its

[1] See discussions of nitrogen balance and "nitrogen lag" by Atwater and associates in the publications of the Office of Experiment Stations, U. S. Department of Agriculture, *e. g.* Bul. 44, pp. 35, 36; Bul. 53, pp. 45, 46; Bul. 69, pp. 23, 24.

[2] BECHER : Studien uber Respiration, Zurich, 1855.

[3] VOIT: Physiologische-Chemische Untersuchungen, Augsburg, 1857, p. 42. Quoted by Graffenberger, Zeitschrift für Biologie, 1892, xxviii, p. 318.

[4] FORSTER: Zeitschrift für Biologie, 1873, ix, p 383.

[5] PANUM : Nordiskt medicinskt Arkiv, 1874, vi, 12. Quoted by Graffenberger, *loc. cit.*

[6] FALCK : Beitrag zur Physiologie, Pharmacologie und Toxicologie, Stuttgart, 1875. Quoted by Graffenberger, *loc. cit.*

[7] FEDER : Zeitschrift für Biologie, 1881, xvii, p. 531.

maximum in about three hours, in others only in about twelve or fourteen hours after the ingestion of the food. As the amounts of proteid thus ingested were often enormous and the meals preceded and followed by more or less prolonged periods of fasting, it is evident that the conditions of nutrition were not always entirely normal.

Moreover, as no account was taken of the usual course of the nitrogen excretion during the corresponding periods of the day, the experiments could at best give only qualitative results. The same is true of the more recent experiments of Rjazantseff,[1] Tschlenoff,[2] and Veraguth[3] who have sought to connect the increased excretion of nitrogen with the work of digestion. Rosemann[4] has made an extensive investigation of the course of the nitrogen excretion during the day, and his pupil Roeske[5] has studied the excretion of phosphoric acid in a similar manner, but in neither case has any direct light been thrown on the " lag " of the excretion studied.

Graffenberger[6] studied the time required for the elimination of the extra nitrogen metabolized after the ingestion of fibrin, gelatin, peptone and asparagin. In each case the subject was kept on a fixed diet for six days and the urine for each day collected in five periods of two, and one of fourteen hours. On the morning of the fourth day the subject took in addition to his regular breakfast so much of the material to be tested as contained five grams of nitrogen. The excretion of this day was compared with the average of the two preceding and two following days. In all cases the excretion seems to have regained the normal at the end of twenty-four hours, but without the appearance in the urine of an amount of nitrogen at all corresponding with that ingested. In the case of the fibrin only 49.2 per cent of the nitrogen was recovered and in the case of the gelatin only 37.6 per cent. These discrepancies are so great as to deprive the results of anything more than a qualitative value, and as neither the food nor fæces was analyzed, it is impossible to judge what became of the remainder of the nitrogen.

[1] RJAZANTSEFF : Archives des sciences biologiques, 1896, iv. p. 393 : Jahresbericht der Thier-Chemie, 1896, p. 349.
[2] TSCHLENOFF : Correspondenzblatt für schweizerische Aertzte, Basel, 1896, 3.
[3] VERAGUTH : Journal of physiology, 1897, xxi, p. 112.
[4] ROSEMANN : Archiv f. d. ges. Physiologie, 1896, lxvi, p. 343.
[5] ROESKE : Ueber den Verlauf der Phosphorsäure Ausscheidung beim Menschen. Dissertation, Griefswald, 1897.
[6] GRAFFENBERGER : Zeitschrift für Biologie, xxviii, 1892, p. 318.

Any considerable increase in the elimination of nitrogen due to muscular exertion seems to appear after a longer interval than when due to extra food. This might be inferred from the work of Parkes,[1] North,[2] Argutinsky,[3] Paton[4] and Krummacher[5] and it is strongly confirmed by more recent experiments. Atwater, Woods, and Benedict[6] in an experiment in which the metabolism of nitrogen (on a uniform diet) was materially increased by active muscular work, lasting eight hours per day, during a period of three days following almost absolute rest, found an apparent lag of a day both at the beginning and at the end of the work period. Dunlop, Paton, et al.,[7] in experiments in which the subject was kept for seven days on a uniform diet and on the fourth day was subjected to muscular exertion about as severe and prolonged as he could well endure, found that the greatest excess in the excretion of nitrogen came sometimes on the day following the exertion and sometimes not until the second day following.

The experiments of Garratt,[8] while not sufficiently controlled to afford very conclusive results, are interesting in indicating that the excretion of sulphates follows a very different course from that of urea. If this conclusion should prove to be correct, investigations in which the nature of the material metabolized is inferred from the relative proportions of these constituents in the urine (notably the very interesting experiments of Kolpakcha[9]) would lose much of their value.

GENERAL DESCRIPTION OF EXPERIMENTS.

Purpose and Plan. — The purpose of the experiments here reported was to study the time of appearance, and the extent and duration

[1] PARKES: Proceedings Royal Society, London, xvi, 1862, p. 45.

[2] NORTH: *Ibid.*, London, xxxvi, 1882, p. 14.

[3] ARGUTINSKY: Archiv f. d. ges. Physiologie, xlvi, 1889, p. 579.

[4] PATON: Reports of the Laboratory of the Royal College of Physicians, Edinburgh, 3, p. 247.

[5] KRUMMACHER: Zeitschrift für Biologie, xxxiii, p. 119.

[6] ATWATER, WOODS, and BENEDICT: Bul. 44, Office of Experiment Stations, U. S. Department of Agriculture.

[7] DUNLOP, PATON, STOCKMAN, and MACCADAM: Journal of physiology, 1897, xxii, p. 69.

[8] GARRATT: Journal of physiology, 1898, xxiii, p. 150.

[9] KOLPAKCHA: Phiziologicheskii Sborink, Charkoff, 1, p. 56; U. S. Department of Agriculture, Office of Experiment Stations, Bul. 45, pp. 308-311, 321-324.

of some of the changes in the urinary excretion which result from the ingestion of a rather large quantity of proteid food when the body is kept in a uniform and perfectly normal condition of nutrition. The diet furnished about 15 grams of nitrogen and 2600 calories of energy and was divided into three nearly equal meals taken at about the ordinary intervals. After the diet had been maintained until the elimination of nitrogen became fairly regular, most of the fat of a single morning meal (July 20th) was replaced by an isodynamic amount of protein, so that about ten grams of extra nitrogen was taken without any change in the supply of potential energy available to the body. When the effect of this change seemed to have disappeared a similar pair of experiments was made in which the protein was simply added to a morning meal (July 22nd) thus increasing the nitrogen about ten grams and the fuel value about 400 calories. The urine was collected in three-hour periods during the day and a nine-hour period at night.

Thus the effect of the extra protein could be followed quite closely for fifteen hours after its ingestion, during which time, as will be seen, the principal effects were produced. In addition to that of nitrogen the determination of sulphur trioxide and phosphorous pentoxide was undertaken. The fæces were also collected and analyzed, and the heat of combustion of the mixed urine of each day was determined.

Subjects and Conditions. — The subjects of these experiments were two young men (the authors) in perfect health, free from any apparent peculiarities as regards digestion or nutrition, and unaccustomed to the use of drugs of any kind or of stimulants or narcotics. Their weights in light summer clothing, were approximately (S.) 140 and (H.) 132 pounds, and were practically the same at the end as at the beginning of the experiments. Before commencing the experiment proper one of the subjects (H.) put himself upon the diet for several days, the other for only a few meals. During the experiments all the meals were taken in the laboratory building and care was exercised to avoid as far possible any special excitement or intense muscular exertion. The work done was of the nature to which the subjects were accustomed, but was somewhat more confining. Although the time covered by the experiments was in the latter half of July the weather throughout was cool and pleasant, as well as clear.

Diet and Routine. — The subjects usually arose at about 5.45 A. M.

and reached the laboratory half an hour later. At 6.30 the bladder was emptied and breakfast immediately begun. This meal consisted of 100 grams "soda-crackers," 660 grams skimmed milk and 50 grams butter. The second meal was taken at 12.30 and the third at 6.30 P. M. These were identical and differed from the breakfast only in containing 25 grams of butter instead of 50 grams. Thus each meal furnished the same amount of nitrogen. No tea nor coffee was taken during the experiment. The simplicity of the diet did not cause it to become distasteful and the amount seemed to be suited to the needs of the subjects. After breakfast the work of the day was begun. One of the subjects (H.) devoted practically the entire day until 5 P. M., and usually the evening from 7 till 9.30, to the analytical work connected with the experiments. Between 5 and 6.30 in the afternoon he walked about a mile and practised vocal exercises for about an hour. The other subject usually took for exercise only a half-mile walk in the evening, spending most of the time from 7 A. M. till 9.30 P. M. either on the analytical work connected with these experiments or in preparing for publication the results of some previous investigations.

The substitution of protein for fat in the breakfast of the fourth day (July 20th) was accomplished by omitting the 50 grams of butter and taking instead 182.6 grams of pulverized, partially dried lean beef, that amount having been found by analysis to possess the same available fuel value as the 50 grams of butter. An amount of salt equivalent to that contained in the butter was added to the beef.

On the sixth day of the experiment (July 22nd) the same amount (182.6 grams) of the beef was simply added to the regular breakfast.

The crackers, butter and beef were prepared in quantity in advance, thus insuring uniformity of composition. The milk was the mixed product of a small local herd, skimmed by means of a centrifugal separator. It was sampled as delivered daily, nitrogen being determined in each sample and in a composite sample representing the entire period. The results were practically uniform at 0.48 per cent. The crackers were also analyzed and found to contain 1.84 per cent of nitrogen. The nitrogen content of the beef, assumed from previous analyses of the same lot, was 5.58 per cent. The amount consumed in each case furnished, therefore, 10.19 grams of nitrogen.

The urine was collected at 6.30 A. M. (immediately before break-

fast) and at intervals of three hours thereafter, until 9.30 P. M. Thus the three meals were taken at the beginnings of the first, third, and fifth periods respectively. The bladder was emptied by natural means in order to avoid any possible irritation which might have resulted from the use of a catheter. As soon as possible after cooling to room temperature the urine was weighed, its specific gravity taken and the analysis begun.

Some lampblack was taken with the first meal of each period in order to color the fæces so that they could be separated from those belonging to preceding meals. The movements were a little slow at times but always entirely normal.

Analytical Methods. — The foods were prepared for analysis and the nitrogen and heat of combustion determined according to the usual methods.[1] From the data thus obtained the fuel value (allowing for the incomplete combustion of proteids in the body) is readily calculated.

Fæces were heated in a water oven until air-dry, then ground, and the nitrogen and heat of combustion determined as in the foods.

In the urine nitrogen was determined by the Kjeldahl method, SO_3 gravimetrically by precipitation with barium chloride, after heating the urine with HCl to decompose etherial sulphates, and P_2O_5 by titration with uranium acetate. The determinations were usually made in duplicate, and the results still further controlled by preparing and analyzing a composite sample representing the urine of the entire day. Heat of combustion of urine was determined by evaporating on filter blocks at 60° and burning in the bomb calorimeter in the usual manner.

GENERAL RESULTS.

The detailed data determined for the urine of each subject during each period are shown in Table A, page 32.

The excretion of nitrogen and SO_3 are graphically represented in Fig. 1, in which the actual weights of the constituents excreted per hour are shown on the ordinates, while the abscissæ mark periods of time. The curves representing the excretion of nitrogen and SO_3 are drawn respectively in solid and broken lines.

[1] See Bul. 69, Office of Experiment Stations, U. S. Department of Agriculture.
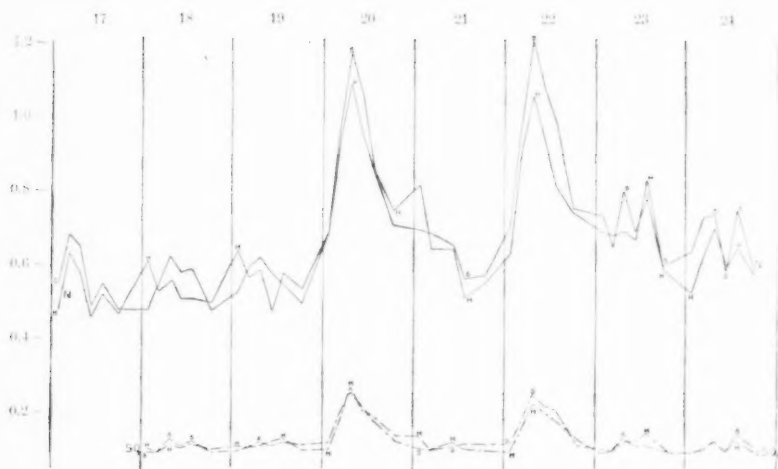
FIG. 1. The curves here shown represent the actual excretion of nitrogen and SO₃ for each subject. The rate of excretion in grams per hour is shown at the left, while the days are shown at the top of the figure. The excretion of nitrogen is represented by solid, and that of SO₃ by broken lines. Where the individual curves show any appreciable divergence they are marked with the initials of the subjects (H. and S.) the perpendicular lines, marking the beginnings of the 20th and 22d, show the points at which the extra protein was consumed. The peculiar form of the nitrogen curve for the first day of the experiment may perhaps be due to the change in routine.

A comparison of the excretions of the two subjects as shown either in the tables or in the curve reveals a very satisfactory agreement throughout. For convenience of discussion, therefore, we have averaged the results, thus permitting the excretion of each constituent to be summarized in a single compact table. Tables B and C show respectively the average quantities of nitrogen and SO₃ excreted. Table D shows the P₂O₅ excretion of " S." Unavoidable delays in the analysis of the urine of " H." resulting in the deposition of phosphates in some of the samples, prevented us from obtaining reliable determinations of P₂O₅ for this subject.

TABLE A.

Amount and Composition of Urine Excreted.

Subject.	Date (1899).	Period.	Amount (Grams).	Specific Gravity.	Nitrogen.		SO_3		P_2O_5	
					Per cent.	Grams.	Per cent.	Grams.	Per cent.	Grams.
H.	July 17	6.30–9.30 A.M.	87.5	1.029	1.64	1.412				
	" 17	9.30 A.M.—12.30 P.M.	266.5	1.012	0.702	1.871				
	" 17	12.30–3.30 P.M.	171	1.0145	0.995	1.701				
	" 17	3.30–6.30 P.M.	82.8	1.028	1.64	1.358				
	" 17	6.30–9.30 P.M.	115	1.026	1.33	1.530				
	" 17 and 18	9.30 P.M.—6.30 A.M.	268.2	1.0275	1.549	4.154				
		Total . . .	991	. . .	. . .	12.026				
S.	July 17	6.30–9.30 A.M.	105.7	1.027	1.535	1.622				
	" 17	9.30 A.M.—12.30 P.M.	358.5	1.0075	0.55	1.972				
	" 17	12.30–3.30 P.M.	374.3	1.008	0.517	1.933				
	" 17	3.30–6.30 P.M.	104	1.021	1.381	1.436				
	" 17	6.30–9.30 P.M.	145.2	1.0205	1.129	1.639				
	" 17 and 18	9.30 P.M.—6.30 A.M.	395	1.020	1.075	4.246				
		Total . . .	1482.7	. . .	. . .	12.848				

H.	July 18	6.30—9.30 A.M.	208.5	1.016	0.840	1.793	0.1408	0.2836	0.121	0.111
	„ 18	9.30 A.M.—12.30 P.M.	127.4	1.026	1.226	1.562	0.2005	0.2968	0.182	0.251
	„ 18	12.30—3.30 P.M.	123.2	1.0225	1.353	1.667	0.2466	0.3284	0.144	0.387
	„ 18	3.30—6.30 P.M.	86.5	1.030	1.725	1.492	0.347	0.3968	0.276	0.463
	„ 18	6.30—9.30 P.M.	99.6	1.030	1.520	1.479	0.3501	0.3587	0.369	0.598
	„ 18 and 19	9.30 P.M.—6.30 A.M.	302.4	1.0275	1.468	4.439	0.2862	0.848	0.311	1.296
		Total	947.6			1242		2.409		3.076
		Composite . . .	947.6	1.025	1.324	1.254	0.2517	2.385		
S.	July 18	6.30—9.30 A.M.	116.3	1.025	1.21	1.407	0.2293	0.229	0.121	0.111
	„ 18	9.30 A.M.—12.30 P.M.	154.1	1.021	1.064	1.638	0.1714	0.242	0.152	0.251
	„ 18	12.30—3.30 P.M.	268.5	1.012	0.682	1.831	0.1392	0.358	0.144	0.387
	„ 18	3.30—6.30 P.M.	167.8	1.075	1.026	1.721	0.180	0.304	0.276	0.463
	„ 18	6.30—9.30 P.M.	186.6	1.018	0.928	1.750	0.1888	0.360	0.369	0.598
	„ 18 and 19	9.30 P.M.—6.30 A.M.	417	1.025	1.027	4.283	0.206	0.846	0.311	1.296
		Total	1312.3			12.630		2.384		3.076
		Composite . . .	1312.3	1.019	0.955	1.253	0.1821	2.389		

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TABLE A (continued).

Subject.	Date (1899).	Period.	Amount (Grams).	Specific Gravity.	Nitrogen. Per cent.	Nitrogen. Grams.	SO₃. Per cent.	SO₃. Grams.	P₂O₅. Per cent.	P₂O₅. Grams.
H.	July 19	6.30–9.30 A.M.	240.2	1.0135	0.7815	1.877	0.1408	0.3305		
	" 19	9.30 A.M.–12.30 P.M.	141	1.026	1.187	1.674	0.2088	0.244		
	" 19	12.30–3.30 P.M.	251.8	1.012	0.690	1.737	0.1300	0.3272		
	" 19	3.30–6.30 P.M.	80.3	1.031	1.753	1.408	0.4375	0.3513		
	" 19	6.30–9.30 P.M.	108.7	1.030	1.583	1.721	0.371	0.3882		
	" 19 and 20	9.30 P.M.–6.30 A.M.	358.3	1.0225	1.335	4.783	0.2455	0.8795		
		Total	1180.3	. . .	. . .	13.200	. . .	2.571		
		Composite .	1180.3	1.0205	1.136	13.40	0.2138	2.524		
S.	July 19	6.30–9.30 A.M.	172.9	1.018	0.900	1.556	0.1690	0.2922	0.103	0.178
	" 19	9.30 A.M.–12.30 P.M.	183.8	1.0195	0.972	1.787	0.1662	0.3054	0.196	0.360
	" 19	12.30–3.30 P.M.	291.2	1.011	0.630	1.834	0.1281	0.3731	0.142	0.415
	" 19	3.30–6.30 P.M.	151.4	1.0205	1.130	1.711	0.2202	0.3183	0.288	0.436
	" 19	6.30–9.30 P.M.	185.1	1.0195	0.885	1.638	0.1967	0.3641	0.273	0.504
	" 19 and 20	9.30 P.M.–6.30 A.M.	470.9	1.0185	0.955	4.497	0.2126	1.0010	0.281	1.320
		Total	1455.3	. . .	. . .	13.023	. . .	2.654		3.213
		Composite .	1455.3	1.018	0.892	1298	0.1719	2.501		

H.

July 20	6.30—9.30 A.M.	216.8	1.0185	0.945	2.049	0.178	0.289		0.219
„ 20	9.30 A.M.—12.30 P.M.	199.7	1.026	1.390	2.776	0.289	0.570	0.253	0.491
„ 20	12.30—3.30 P.M.	310.9	1.06	1.033	3.212	0.2589	0.849	0.239	0.650
„ 20	3.30—6.30 P.M.	263.4	1.019	1.071	2.821	0.2355	0.6263	0.213	0.583
„ 20	6.30—9.30 P.M.	171.9	1.024	1.47	2.527	0.2362	0.579	0.162	0.428
„ 20 and 21	9.30 P.M.—6.30 A.M.	557.3	1.0175	1.192	6.43	0.2314	1.2800	0.258	1.331
	Total	1720	. . .	. . .	20.028	. . .	4.129	. . .	3.702
	Composite .	1720	1.019	1.168	2.010	0.2298	3.902	. . .	. . .

I.

July 20	6.30—9.30 A.M.	210.3	1.019	0.967	2.034	0.1676	0.323	0.104	0.323
„ 20	9.30 A.M.—12.30 P.M.	193.7	1.026	1.486	2.878	0.307	0.599	0.253	0.491
„ 20	12.30—3.30 P.M.	272.2	1.0215	1.277	3.476	0.280	0.780	0.239	0.650
„ 20	3.30—6.30 P.M.	240.3	1.022	1.310	3.148	0.241	0.687	0.213	0.583
„ 20	6.30—9.30 P.M.	263.7	1.017	0.985	2.518	0.204	0.443	0.162	0.428
„ 20 and 21	9.30 P.M.—6.30 A.M.	515	1.019	1.231	6.340	0.204	1.330	0.258	1.331
	Total	1695.2	. . .	. . .	20.394	. . .	4.061	. . .	
	Composite .	1695.2	1.020	1.202	20.37	0.2320	3.933	. . .	

TABLE A (continued).

Subject.	Date (1899).	Period.	Amount (Grams).	Specific Gravity.	Nitrogen.		SO₃.		P₂O₅.	
					Per cent.	Grams.	Per cent.	Grams.	Per cent.	Grams.
H.	July 21	6.30–9.30 A.M.	208.4	1.019	1.165	2.428	0.1960	0.4085		
	" 21	9.30 A.M.–12.30 P.M.	172.7	1.0185	1.120	1.934	0.1728	0.2984		
	" 21	12.30–6.30 P.M.	320.7	1.018	1.206	3.868	0.2308	0.7403		
	" 21	6.30–9.30 P.M	88.2	1.030	1.725	1.521	0.3843	0.3390		
	" 21 and 22	9.30 P.M.–6.30 A.M.	330.6	1.023	1.504	4.972	0.2729	0.9024		
		Total . . .	1120.6	. .	. .	14.723	. .	2.689		
S.	July 21	6.30–9.30 A.M.	181.9	1.018	1.148	2.088	0.1752	0.3186	0.102	0.185
	" 21	9.30 A.M.–12.30 P.M.	146.2	1.0225	1.391	2.034	0.2066	0.3021	0.207	0.303
	" 21	12.30–6.30 P.M.	326.5	1.0185	1.193	3.895	0.2113	0.6899	0.277	0.904
	" 21	6.30–9.30 P.M.	117.5	1.027	1.442	1.694	0.3091	0.3631	0.405	0.476
	" 21 and 22	9.30 P.M.–6.30 A.M.	392.5	1.022	1.311	5.142	0.2703	1.0008	0.311	1.223
		Total . . .	1164.6	. .	. .	14.853	. .	2.734	. .	3.091
		Composite .	1164.6	1.0205	1.274	14.84	0.2290	2.665	. .	. .

H.

July 22 6.30–9.30 A.M.	119	1.0275	1.595	1.898	0.2400	0.2855		0.159
„ 22 9.30 A.M.–12.30 P.M.	144.7	1.0255	1.650	2.718	0.264	0.4881	0.103	0.351
„ 22 12.30–3.30 P.M.	179.2	1.027	1.750	3.136	0.3810	0.6827	0.170	0.654
„ 22 3.30–6.30 P.M.	157.2	1.028	1.770	2.782	0.3937	0.6490	0.270	0.647
„ 22 6.30–9.30 P.M.	132	1.0285	1.845	2.435	0.4227	0.5579	0.310	0.597
„ 22 and 23 9.30 P.M.–6.30 A.M.	374.4	1.026	1.820	6.814	0.3198	1.1974	0.329	1.283
Total	1126.5			19.783		3.831	0.305	3.691
Composite	1126.5	1.027	1.749	19.70	0.3357	3.789		

S.

July 22 6.30–9.30 A.M.	154.7	1.0225	1.370	2.119	0.2286	0.3535		
„ 22 9.30 A.M.–12.30 P.M.	206.4	1.023	1.407	2.910	0.2643	0.5393		
„ 22 12.30–3.30 P.M.	242.3	1.0245	1.487	3.610	0.3160	0.7655		
„ 22 3.30–6.30 P.M.	208.9	1.0255	1.544	3.217	0.3221	0.6725		
„ 22 6.30–9.30 P.M.	181.4	1.0275	1.635	2.957	0.3564	0.6465		
„ 22 and 23 9.30 P.M.–6.30 A.M.	420.6	1.023	1.610	6.772	0.2546	1.0705		
Total	1414.3			21.585		4.048		
Composite	1414.3	1.024	1.536	21.72	0.2580	4.030		

TABLE A (*continued*).

Subject.	Date (1899).	Period.	Amount (Grams).	Specific Gravity.	Nitrogen.		SO₃		P₂O₅	
					Per cent.	Grams.	Per cent.	Grams.	Per cent.	Grams.
H.	July 23	6.30—9.30 A.M.	116.2	1.025	1.774	2.061	0.2605	0.3027		
	" 23	9.30 A.M.—12.30 P.M.	113	1.026	1.816	2.052	0.2801	0.3165		
	" 23	12.30—3.30 P.M.	111.9	1.031	1.840	2.059	0.3581	0.4007		
	" 23	3.30—6.30 P.M.	100.7	1.031	1.987	2.001	0.3889	0.3916		
	" 23	6.30—9.30 P.M.	148.5	1.0265	1.677	2.490	0.3150	0.4678		
	" 23 and 24	9.30 P.M.—6.30 A.M.	303	1.029	1.753	5.312	0.2867	0.8686		
		Total	893.3	. . .	. . .	15.975	. . .	2.7479		
		Composite .	893.3	1.0295	1.786	15.95	0.3102	2.770		
ƒ.	July 23	6.30—9.30 A.M.	123.3	1.025	1.788	2.204	0.2666	0.3287	0.145	0.179
	" 23	9.30 A.M.—12.30 P.M.	116.1	1.026	1.693	1.966	0.2714	0.3152	0.285	0.331
	" 23	12.30—3.30 P.M.	168	1.0265	1.438	2.416	0.2540	0.4267	0.251	0.422
	" 23	3.30—6.30 P.M.	126.4	1.025	1.640	2.073	0.2875	0.3634	0.327	0.413
	" 23	6.30—9.30 P.M.	147.7	1.028	1.563	2.309	0.2470	0.3649	0.361	0.532
	" 23 and 24	9.30 P.M.—6.30 A.M.	354.4	1.0235	1.518	5.380	0.2547	0.9028	0.38	1.198
		Total	1035.9	. . .	. . .	16.351	. . .	2.7017	. . .	3.075
		Composite .	1035.9	1.0245	1.568	16.24	0.2590	2.683	. . .	. . .

H.	July 24	6.30—9.30 A.M.	94.2	1.0305	1.801	1.583	0.3495	0.3810
	:: 24	9.30 A.M.—12.30 P.M.	103.3	1.030	1.823	1.883	0.3270	0.3378
	:: 24	12.30—3.30 P.M.	102.0	1.030	2.075	2.116	0.3971	0.4054
	:: 24	3.30—6.30 P.M.	79.7	1.0305	2.255	1.797	0.4171	0.3324
	:: 24	6.30—9.30 P.M.	92.0	1.0335	2.116	1.947	0.4334	0.3987
	:: 24 and 25	9.30 P.M.—6.30 A.M.	296.9	1.029	1.770	5.219	0.3028	0.8989
		Total . .	768.1	. .	. .	14.545	. .	2.6712
		Composite .	768.1	1.0305	1.913	1.469	0.367	2.463
S.	July 24	6.30—9.30 A.M.	142.0	1.021	1.364	1.937	0.2305	0.2989
	:: 24	9.30 A.M.—12.30 P.M.	100.2	1.025	1.361	2.180	0.2113	0.3385
	:: 24	12.30—3.30 P.M.	157.5	1.025	1.425	2.244	0.2589	0.4077
	:: 24	3.30—6.30 P.M.	106.6	1.027	1.650	1.759	0.3116	0.3322
	:: 24	6.30—9.30 P.M.	177.0	1.025	1.274	2.255	0.2624	0.444
	:: 24 and 25	9.30 P.M.—6.30 A.M.	394.3	1.0215	1.348	5.315	0.2411	0.9517
		Total . . .	1137.6	. .	. .	15.690	. .	2.7594
		Composite .	1137.6	1.023	1.367	1.535	0.2433	2.770
H.	July 25	Total . .	804.7	. . .	1.781	14.30		
S.	:: 25	::	1135.0	. . .	1.342	15.50		
H.	:: 26	::	789.8	. . .	1.774	14.64		
H.	:: 27	::	858.6	. . .	1.649	14.16		

TABLE B.

Average Nitrogen Excretion by Periods.

(Expressed in grams.)

Period.[1]	17th	18th	19th	20th	21st	22d	23d	24th
I	1.52	1.60	1.71	2.04	2.26	2.01	2.13	1.76
II	1.92	1.60	1.73	2.83	1.98	2.81	2.01	2.03
III	1.82	1.75	1.78	3.34	3.88	3.37	2.24	2.18
IV	1.40	1.61	1.56	2.99		3.00	2.04	1.78
V	1.58	1.62	1.68	2.52	1.61	2.70	2.40	2.10
VI	4.20	4.36	4.64	6.49	5.06	6.79	5.35	5.27
Total	12.44	12.54	13.10	20.21	14.79	20.68	16.17	15.12

[1] The Roman numerals here designate the experimental periods into which each day was divided, viz. five three-hour periods the first beginning at 6.30 A. M., the last ending at 9.30 P. M., and being followed by a nine-hour period (VI) lasting from 9.30 P. M. until 6.30 A.M. of the next calendar day. The meals containing the extra protein were taken at the beginnings of the 20th and 22d respectively.

In these tables the increased excretion of each of the three constituents following the ingestion of the beef can readily be seen.

In order to bring out the *relative* variations in the rate of excretion of the different constituents we have plotted the average results in the curves shown in Figs. 2 and 3, in which the abscissæ, as in Fig. 1, represent periods of time while the ordinates represent relative variations expressed in per cents of an assumed average rate of excretion, viz., for nitrogen 0.5 gram per hour and for SO_3 and P_2O_5 0.1 gram per hour. (In reality these figures are somewhat lower than the average in each case.) The excretion of nitrogen is represented by a solid, that of SO_3 by a broken, and that of P_2O_5 by a dotted line. The curve at the bottom of Fig. 3 represents the

TABLE C.

Average Excretion of SO_3 by Periods.

(Expressed in grams.)

Period.[1]	18th	19th	20th	21st	22d	23d	24th
I	0.283	0.311	0.326	0.364	0.320	0.316	0.300
II	0.266	0.300	0.569	0.300	0.514	0.316	0.338
III	0.351	0.350	0.785	0.715	0.724	0.414	0.406
IV	0.309	0.335	0.640		0.646	0.378	0.332
V	0.352	0.376	0.561	0.351	0.602	0.416	0.432
VI	0.836	0.940	1.211	0.982	1.134	0.886	0.925
Total	2.40	2.61	4.09	2.71	3.94	2.73	2.73
Ratio N : SO_3	100:19.3	100:19.9	100:20.2	100:18.3	100:19.0	100:16.9	100:18.1

[1] See note following table B.

actual quantities of P_2O_5 excreted, being drawn on the same scale as the curves in Fig. 1.

Nitrogen. — Both when the protein is substituted for fat and when it is simply added to the diet, the nitrogen excretion shows a distinct increase in the first three-hour period following the ingestion, in the second period (3 to 6 hours) the increase is more marked, and in the third (6 to 9 hours) the maximum is reached. During the fourth, fifth, and sixth (night) periods the excretion steadily falls, but not so rapidly as it rises during the first nine hours. On the day following that on which the beef was substituted for butter, the excretion is seen to fall constantly toward the normal (though showing something of the ordinary daily fluctuations) until in the fifth period of the second day — 36 to 39 hours after the ingestion of the extra protein — the previous level of nitrogen elimination is regained.

TABLE D.

Excretion of P_2O_5 by Periods.

(Expressed in grams.)

Periods.[1]	18th	19th	20th	21st	22d	23d
I	0.141	0.178	0.219	0.185	0.159	0.179
II	0.281	0.360	0.491	0.303	0.351	0.331
III	0.387	0.415	0.650	0.904	0.654	0.422
IV	0.463	0.436	0.583		0.647	0.413
V	0.508	0.504	0.428	0.476	0.597	0.532
VI	1.296	1.320	1.331	1.223	1.283	1.198
Total	3.08	3.21	3.70	3.09	3.69	3.08

[1] See note following table B.

After the day on which the extra protein was simply added to the diet, the rate of excretion did not fall back to that of the first days but continued sensibly higher throughout the remainder of the experiment. Taking into account this increase, which is discussed beyond in connection with the nitrogen balance, it appears that the immediate effect of the protein ingested had disappeared at the end of 39 hours.

Graffenberger (*loc. cit.*), adding to an otherwise uniform diet enough fibrin to supply 5 grams of nitrogen, found the maximum increase in the third and fourth hours, after which the excretion declined steadily but did not reach the normal until after about 24 hours. In all about one-half the added nitrogen was recovered by Graffenberger in the urine, whereas in the experiments here reported about four-fifths of the added nitrogen was thus recovered. That a longer time was required both to reach the maximum and to regain the normal in
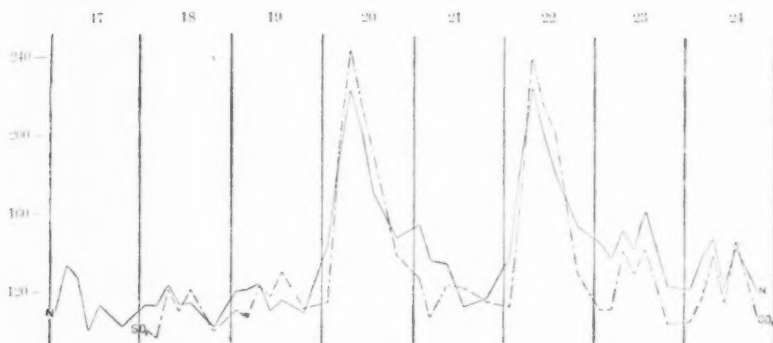
FIG. 2. The curves here shown represent the relative fluctuations in the average rates of excretion of nitrogen and SO_3. The values on the left represent percentages of an assumed standard rate of excretion for each of these constituents. The use of solid and broken lines and the representation of periods of time are the same as in Fig. 1. It will be seen that in general the excretion of sulphates ran quite closely parallel to that of nitrogen. The divergences which occur are discussed in the text.

our experiments is probably due to the much larger amount of nitrogen metabolized.

Sulphates. — In general the excretion of sulphates ran closely parallel to that of nitrogen both on the normal days and on those affected by the extra ingestion of protein. On close examination, however, it appears that the relative increase of SO_3 as compared with that of nitrogen is somewhat less in the first period following the ingestion and a little greater in the third. After reaching the maximum the rate of excretion of the sulphates falls rapidly, reaching the normal after about 27 hours. Hence during the first half of the second day after ingestion of the extra protein, the rate of excretion of sulphates was practically normal while that of nitrogen was still high. This not only appears in the curve but is sufficient to render the ratio of SO_3 to nitrogen sensibly lower on these days than on either of the normal days or those on which the extra protein was taken and the greater part of the extra nitrogen and sulphur eliminated.

In experiments in which the metabolism was increased by muscular work, Garratt (*loc. cit.*) found the increased excretion of sulphates to be proportionate to that of urea but of less duration and of greater
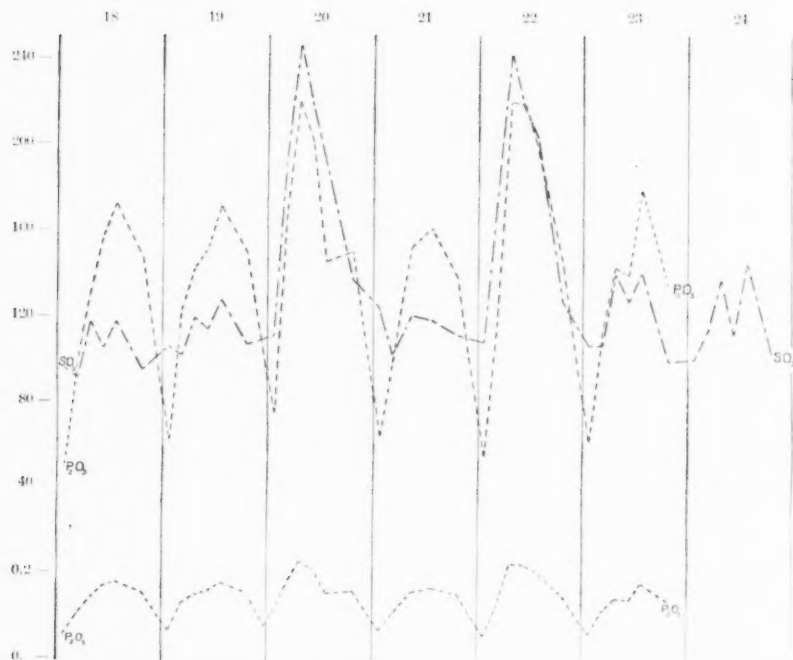
FIG. 3. The upper curves show the relative rates of excretion of SO_3 and P_2O_5 plotted in exactly the same manner as the curves in Fig. 2. The curve near the bottom of the figure represents the actual rate of excretion of P_2O_5 plotted in the same manner as the curves in Fig. 1. It appears that the normal daily course of the excretion of phosphates shows no similarity to that of nitrogen or sulphates, but that the increased elimination following the ingestion of beef occurred practically simultaneously for the three constituents. The phosphates, however, showed a smaller absolute and relative increase and more quickly regained the normal rate of excretion.

intensity, requiring only one-half as long either to reach the maximum or to regain the normal. Our results fail to reveal any such difference in the form of the curves, although the slight variations already noted are in the direction indicated by Garratt. Here again we have an indication that the "lag" may be different according as the metabolism is stimulated by food or by muscular exertion.

Phosphates. — The course of the phosphate excretion during the normal days did not run parallel to that of nitrogen as did the sul-

phates. The rate of excretion during the different periods of the day varied to an immensely greater extent than did that of either of the other constituents, and also differed from them in showing (as measured by three-hour periods) only one rise and one fall in 24 hours while nitrogen and sulphates show two rises and two falls. The fall in the phosphate excretion continues not only during the night but also into the first period of the morning, during which the nitrogen and sulphates tend normally to rise. Roeske (*loc. cit.*), studying the excretion of P_2O_5 by two-hour periods, found that the minimum occurred most often in the morning, usually in the first but occasionally in the second or third period.

The ingestion of beef caused in the first case a very slight rise in the first period and a marked one in the second, while the maximum was reached simultaneously with that of nitrogen and sulphates in the third period. After this the fall was rapid and the normal rate of excretion was regained by the twelfth hour. After the second ingestion of beef the excretion of phosphates was unaffected in the first period and nearly so in the second, but rose rapidly to a maximum in the third and then fell more slowly reaching the normal about fifteen hours after the ingestion, while on the day following, the excretion of phosphates like that of nitrogen and sulphates was somewhat irregular. Thus the increase in phosphates bears the same relation to that of sulphates as the latter does to the increased excretion of nitrogen, beginning apparently a little later, reaching the maximum at about the same time and regaining the normal appreciably earlier.

Heat of Combustion of Urine. — It is well known that the heat of combustion of urine is always higher than would be the case if the urine consisted simply of water, salts, and a sufficient amount of urea to account for the nitrogen present. The excess is due in part of course to non-nitrogenous organic matter and in part to nitrogenous compounds less highly oxidized and containing more potential energy than urea, *e. g.* hippuric acid and nitrogenous extractives.

We should expect that the ratio of nitrogen to heat of combustion in the urine would vary somewhat with the nature of the proteid ingested. A special investigation of the subject is contemplated in this laboratory. The change in ratio noted below is believed to be chiefly due to the sudden increase in proteid metabolism rather than to the partial change in the nature of the protein consumed.

In the composite samples of urine representing the total excretion

for each day we have determined the heat of combustion and calcu-
lated its relation to the nitrogen excretion. The results are shown
in Table E. The last column of this table shows for each day the
number of calories eliminated in excess of what would be yielded by
an amount of urea corresponding to the total nitrogen present.

TABLE E.

Relation of Nitrogen to Heat of Combustion in Urine.

Subject.	Date, 1899.	Urine excreted. Grams.	Heat of combustion per gram. Calories (small).	Total heat of combustion of Urine. Calories (large).	Nitrogen excreted. Grams.	Ratio of Nitrogen to Calories.	"Calories in excess of Urea."[1]
H.	July 18	947.6	110.6	104.8	12.54	1 : 8.4	37.1
"	19	1180.3	99.4	117.3	13.40	1 : 8.8	44.9
"	20	1720.0	89.2	153.5	20.10	1 : 7.6	45.0
"	21	1120.6	100.7	112.9	13.62	1 : 8.3	39.4
"	22	1126.5	136.7	154.0	19.70	1 : 7.8	47.6
"	23	893.3	145.9	130.3	15.95	1 : 8.2	44.2
"	24	768.1	155.2	119.2	14.69	1 : 8.1	39.9
S.	July 18	1312.3	83.7	109.8	12.53	1 : 8.8	42.1
"	19	1455.3	76.3	110.2	12.98	1 : 8.5	40.1
"	20	1695.2	90.1	152.7	20.37	1 : 7.5	42.7
"	21	1164.6	101.9	118.7	14.84	1 : 8.0	38.6
"	22	1414.3	114.6	162.0	21.72	1 : 7.5	44.7
"	23	1035.9	130.0	134.7	16.24	1 : 8.3	47.0
"	24	1137.6	104.1	118.4	15.55	1 : 7.6	34.4

[1] See explanation of this column p. 47. In the calculation the heat of combustion of
urea is taken as 2.53 (large) calories per gram.

It appears that when the excretion of nitrogen rises in con-
sequence of an increased ingestion of protein the heat of combustion
also rises but to a less extent, so that the ratio of nitrogen to calories

of combustion is altered, the effect being greatest on the days on which the greatest elimination of nitrogen takes place.

When we calculate the heat of combustion of a quantity of urea equivalent to the nitrogen present and deduct it from the total heat of combustion of the urine eliminated, the "calories in excess of urea," which evidently correspond to the amounts of less highly oxidized organic matter, appear to be fairly constant for the different days and quite independent of the fluctuations of the nitrogen excretion. It seems probable that this point will repay further investigation.

Nitrogen Balance. — Table F shows the income and outgo of nitrogen for each subject.

TABLE F.

Income and Outgo of Nitrogen.

Subject.	Period of days.	Duration in days.	Nitrogen. (In grams.)				
			In Food.	In Urine.	In Fæces.	Gain or Loss.	Average Gain or Loss per day.
H.	First [1]	3	45.06	37.65	2.31	+ 5.10	+1.70
	Second [2]	2	40.23	34.75	2.32	+ 3.16	+1.58
	Third [3]	3	55.25	50.31	2.58	+ 2.36	+0.78
	Fourth [4]	3	45.06	42.50	2.91	− 0.35	−0.12
	Total .	11	185.60	165.21	10.12	+10.27	+0.93
S.	First [1]	3	45.06	40.34	1.84	+2.88	+0.96
	Second [2]	2	40.23	35.24	2.11	+2.88	+1.44
	Third [3]	3	55.25	53.63	2.75	−1.13	−0.38
	Fourth [4]	1	15.02	15.50	1.14	−1.62	−1.62
	Total .	9	155.56	144.71	7.84	+3.01	+0.33

[1] During this period, July 17, 18, 19, the diet was uniform as described on p. 29.

[2] This period begins with the morning of July 20, when protein was substituted for fat in the morning meal, and lasts two days.

[3] This period begins with the morning of July 22, when protein was added to the regular morning meal, and lasts three days.

[4] This period begins three days after the addition of protein, the nitrogen excretion having again become regular.

It was of course impossible to determine the nitrogen balance while the experiment was in progress on account of the delay in obtaining and drying the fæces. Hence of the fluctuations shown in the table all that was known at the time or that would have been ascertained if only the urine had been analyzed, was that the daily elimination of nitrogen tended to rise during the time covered by the experiments. Such an increase might be attributed either (1) to the fact that the work done by the subjects was rather exacting and resulted in slight fatigue as the experiment progressed, or (2) to a slight but prolonged stimulation of metabolism caused by the ingestion of extra protein. On examining the nitrogen balance, however, it appears that during the early days of the experiment the subjects were receiving more nitrogen than was being eliminated, under which circumstances the daily excretion would tend to rise gradually until nitrogen equilibrium was attained. This is apparently just what occurred with " H.," who is seen to have reached nitrogen equilibrium after eight days, while " S." shows first a gain and then a loss of nitrogen, so that in the nine days during which he was under observation he eliminated about as much nitrogen as he ingested.

It should be noted that the two chief sources of error in determining the nitrogen balance were doubtless the elimination of nitrogen through the skin and the loss by volatilization in drying fæces for analysis, both of which would have the effect of indicating a storage of nitrogen in the body, as would also any slight mechanical loss of food after weighing.

In the third period the general features of the curve seem to be alike for the two subjects, although one is here gaining, and the other losing, nitrogen.

SUMMARY.

The experiments were conducted upon two healthy young men under normal conditions of nutrition.

As measured by three-hour periods, the rates of excretion of nitrogen and sulphates run closely parallel and normally show a tendency to rise during the morning, reaching a maximum after the midday meal with a slight fall in the following period and another rise after the evening meal. During the night the excretion usually reaches the minimum.

The excretion of phosphates on the normal days described a curve altogether different from that of nitrogen and sulphates, rising

steadily from the middle of the morning until the time of retiring, then falling during the hours of sleep and continuing to fall for three hours after rising, reaching a minimum after breakfast.

When lean beef sufficient to furnish about 63.7 grams of extra protein was taken with breakfast, the nitrogen began to rise in the first three hours and reached a maximum between the sixth and ninth hours, after which it declined at first rapidly and then more slowly, reaching the normal after about 36 to 39 hours.

The increased excretion of sulphates was proportional to that of nitrogen and followed the same general course. It appeared, however, to begin a little later and certainly regained the normal a little earlier.

The increase in the rate of excretion of phosphates apparently began a little later but reached a maximum at the same time with that of nitrogen, after which it fell rapidly, regaining the normal about 12 to 15 hours after the ingestion of the beef.

The increased heat of combustion of the urine was but little greater than would correspond to an amount of urea equivalent to the extra nitrogen eliminated. This would seem to indicate that the total amount of the less highly oxidized constituents of the urine was but little affected.

The nature and extent of the changes in the urine seem to have been about the same when the protein was simply added to the diet as when it was substituted for an isodynamic amount of fat.

A moderate gain or loss of body nitrogen does not seem to affect the changes noted.
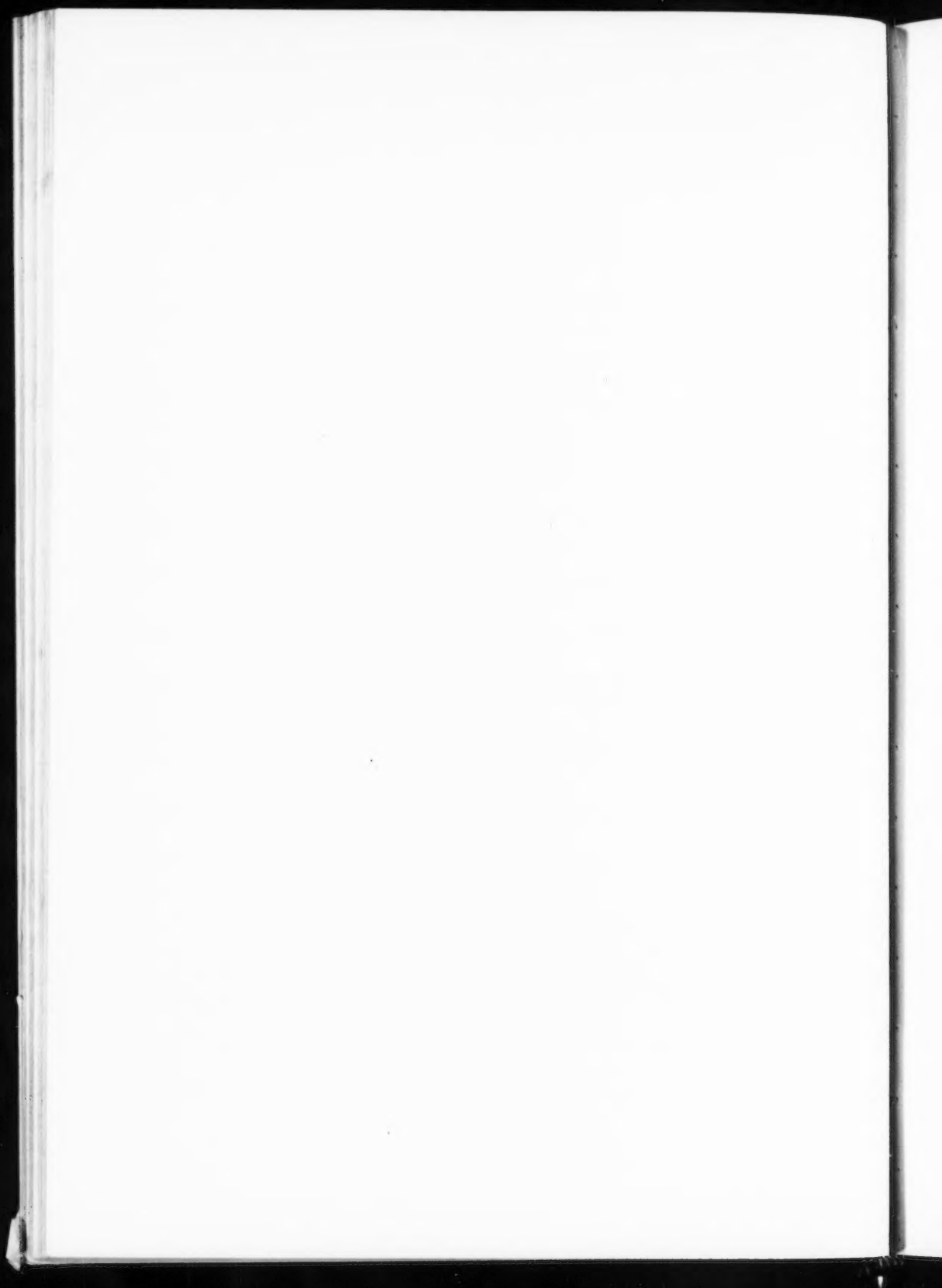
ON CARDIAC THROMBOSIS FOLLOWING COMPLETE REMOVAL OF THE SUPRARENAL GLANDS.

BY B. MOORE AND C. O. PURINTON.

[*From the Physiological Laboratory of the Yale Medical School.*]

A S it is our purpose in this paper to give merely a preliminary account of certain experiments upon suprarenal removal, it is not necessary to enter further upon the history of the subject [1] than to state that all modern observers agree that *complete* suprarenal removal is invariably fatal, and in nearly all cases within a very brief period.

Certain observers have removed both glands in the white rat without causing death, but on post-mortem examination have found a satisfactory explanation of this result in the presence of accessory glands.

Nearly all the animals experimented upon die within a period of one to three days after the removal of the second gland. Thus Brown-Séquard found death to take place usually within twelve hours, Tizzoni was able to keep a small percentage of rabbits in which double suprarenal removal had been performed, alive for a sufficient length of time to show pigmentation of the skin, but the great majority of the animals died very rapidly.

The general rule then is that death takes place with great rapidity in most animals after double suprarenal removal.

The cause of this rapid death still remains obscure, in spite of the vast amount of work which has been done upon the subject. The symptoms described by most authors are almost identical with those of pronounced Addison's disease, such as muscular weakness, loss of appetite, loss of tone of the vascular system, and, as a primary cause of death, paralysis of the respiratory muscles.

Now all these are scarcely sufficient to account for the rapid death which ensues usually within less than twenty-four hours after the operation. In some cases of Addison's disease in man, the suprarenals have been found completely caseous, without a trace of normal tissue, and showing that the normal tissue must have been

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[1] For a résumé of the history, see Schäfer's Textbook of physiology, i. p. 948.

absent for some time preceding death. Death from suprarenal inefficiency slowly brought about does not accordingly take place with that rapidity which we find in animals from which the glands (or the second gland) have been suddenly removed.

Removal of the suprarenals in most instances causes death so rapidly that some observers have claimed that the fatal result is due to surgical shock. That shock is not the cause of death is, however, shown by the fact that removal of the first gland is not often followed by a fatal result, while that of the second is never recovered from save when accessory glands are present. This result is obtained even when the animal is allowed to recover completely from the effects of the first operation, and when accordingly the shock of the second operation would not be any greater than that of the first. This is shown clearly in the experiments of which we here publish an account; we have also obtained evidence that the gland left behind undergoes a compensatory hypertrophy, and that death after the removal of the second gland is probably due to the stoppage of the circulation by cardiac thrombosis occurring during life and after the removal of the second gland.

The experiments have been carried out upon cats and kittens. The cat is a favorable animal for such experimentation for two reasons. In the first place, the muscles of the abdominal wall in the lumbar region are thin, and consequently the operation is not so deep as in many other animals. Secondly, the suprarenals are not so closely attached to or so close to the vena cava as in most animals, and consequently there is less risk of severe hæmorrhage in their removal. For these reasons the operation of suprarenal removal, which is under any circumstances a difficult one, is less formidable in the cat than in most other animals.

We have made no attempt to remove both glands in the same operation, but have allowed the animals to recover completely from the first removal before attempting to remove the second gland. The gland was removed in each case by a lumbar incision parallel to the vertebral column, just above the kidney, which can be very easily felt through the thin abdominal muscles, and somewhat closer to the vertebral column than the kidney; so that the incision lay in the space included between the lowest rib, the vertebral column, and the kidney. After opening the peritoneum, the suprarenal is easily seen crossed by a large vein, which courses over the parietes of the abdomen and passes over the gland to empty into the vena

cava. This vein is one of the obstacles in the operation, since as it opens into the inferior vena cava directly, it bleeds almost as profusely from its central end as the cava itself if it be torn, or cut without ligaturing, during the operation. The peritoneum is detached by tearing with fine forceps near where this vein passes over the gland, a double ligature is passed round the vein peripherally to the gland, and the vein is cut between the ligatures. The gland is next detached by gently tearing with forceps from the abdominal wall and other tissues, except where the large vein passes away from it towards the vena cava. The gland is then pulled forward with the thumb and forefinger and a catgut ligature passed underneath so as to ligature the pedicle artificially formed containing the large vein as above described. The ligature is tied as far from the gland as possible, the ends cut, and then the suprarenal is snipped off with scissors close to the gland. In this manner the gland can be removed in a perfectly complete condition. It must not be touched with forceps throughout the operation, otherwise the capsule becomes broken, and there is no guarantee of complete removal.

The removal is almost equally easy or equally difficult on the two sides, and we have accordingly removed the gland in alternate animals in opposite order. This we have done also in order to eliminate the chance of a difference in size on the two sides, in determining whether any compensatory hypertrophy took place in the case of the gland left behind.

Up to the present we have operated in all upon fifteen cats and four kittens. Of these, three cats died during the first operation, in one case probably from the anæsthetic, and in the other two of hæmorrhage. Three died after the first operation, — one of peritonitis, one of general septicæmia, and one was chloroformed on account of a septic wound. One died from an accident with the anæsthetic in removing the second gland, twenty-nine days after the removal of the first. Four died after removal of the second gland. Four are still alive in which the first gland has been removed for periods of thirty-four, thirteen, eleven, and two days respectively. All four kittens upon which the operation of removal of the first gland has been carried out were alive and in good condition, seventeen days after the operation.

Our chief communication is with regard to two of the four animals which survived both operations. All four of these animals completely recovered from the anæsthetic after the second operation,

and were observed in each case for some hours afterwards. The animal in each case seemed completely to recover from the shock of the operation, purred when stroked, and moved about the room. Yet in all four cases the animal died within twenty-four hours of the operation. In the first two cases no cause of death could be discovered by post-mortem examination; but most attention was paid to the abdomen, and the heart and great vessels were not examined. In the third and fourth case on examining the heart and vessels we were more fortunate in locating the cause of *exitus.* In each case the right auricle contained a large white adherent clot, which was attached to the auriculo-ventricular valve and sent processes toward the ventricle and venæ cavæ. In the fourth case both venæ cavæ were plugged for over two inches with nearly white elastic clots which were firm enough to pull out of the veins from the auricular ends, and these were attached to and formed part of a large clot which nearly filled the right auricle; in addition a large ante-mortem clot was found in the left auricle with processes passing down towards the auriculo-ventricular valves and attached to the chordæ tendineæ.

Microscopic examination showed that the clots were of recent formation, as they presented no appearance of a new formation, and contained chiefly polymorphonuclear leucocytes enclosed in a clear hyaline ground substance. The clots presented all the microscopic appearances of having been formed after the second operation and before death.

The following are the protocols of these two experiments.

Experiment I. — Feb. 17. Left suprarenal gland removed from female cat weighing 1.7 kilograms.

March 3. Right suprarenal also removed; weight of animal now 1.85 kilograms. Operation completed at 1.15 P. M. At 8 P. M. animal seemed well, purred on stroking, and moved about the room. Next morning animal was found dead.

Post-mortem examination. Peritoneum in normal condition, no hæmorrhage, all organs appear normal. Small amount of pericardial fluid. Heart walls are pale colored. Right ventricle almost empty. Left ventricle hard. Both auricles nearly empty. Right auricle contained a clot white in color and attached by processes to auriculo-ventricular valve so as to lie in auriculo-ventricular opening. Histological examination showed no organization in the clot. The clot was tough and elastic. No accessory suprarenal glands were discovered and both glands had been completely removed. The brain appeared normal, and there was no apparent hypertrophy of the pituitary gland. The solar plexus and semilunar ganglia were dissected out and had suffered no extensive injury from the operation.

Experiment II. — March 3. The right suprarenal gland, *weighing 0.280 gram*, was removed from a large male maltese cat weighing 4.4 kilograms.

March 23. The left suprarenal gland, *weighing 0.402 gram.* was removed. Operation finished at 5.35 P. M. ; animal recovered well from anæsthetic and was able to move about at 7.30 P. M. At 9 A. M. next morning the animal had apparently completely recovered from the shock of the operation ; weight of animal 3.7 kilograms. At 2.45 P. M. the animal drank a little milk. Urine passed after operation was examined, reaction alkaline, phosphates abundant, no sugar or albumin present, suprarenal chromogen entirely absent. The animal was observed alive at 5.30 P. M. and at 5.45 P. M. was found dead.

Post-mortem examination. Kidneys slightly congested and blood-vessels of peritoneum somewhat congested near wound, but no general peritonitis or other probable cause of death observable in peritoneal cavity. No hæmorrhage. Right ventricle flaccid and nearly empty of blood. Left auricle and ventricle congested with blood. In right auricle was found a large clot of pale pink color and firm consistency which was connected with similar almost colorless clots five to six centimetres long, which plugged up both venæ cavæ and were further attached by projecting processes to auriculo-ventricular valves. The clots in the veins were very tough and elastic, and were pulled out whole by traction on the auricular ends. Histological examination as before showed no organization, but merely a number of leucocytes in a clear ground substance.

In the second of the two experiments it is interesting to observe that a marked hypertrophy of the remaining gland had taken place, since the right gland which was first removed weighed only 0.280 gram, while the left gland removed twenty days later weighed 0.402 gram. In the other animal, unfortunately, the gland first removed was not weighed, but in another animal which died during the second operation twenty-nine days after the first, and in which the glands were removed in the reverse order, the left gland weighed 0.230 gram, while the right gland weighed 0.322 gram ; showing again a marked hypertrophy. The glands first removed have been carefully weighed in the four animals which we have now under experiment, and we hope to obtain further evidence on the subject of compensatory hypertrophy.[1]

This point is of interest in regard to the view at one time held from the diminution in relative size of the suprarenals after birth that these glands were structures only of importance in the embryo and atrophying considerably after birth, since it directly demonstrates the

[1] Evidence of this hypertrophy has since been obtained in three of these animals, the fourth dying before hypertrophy had much advanced.

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functional activity of the gland in adult life. We also hope with other animals to get additional evidence as to ante-mortem clotting as a cause of death after suprarenal removal; but even if it should turn out not to be a constant consequence of that operation, we hold the occurrence twice of such a phenomenon sufficiently interesting to merit description.[1] The only cause of clotting which has suggested itself to us is closely connected with the action of the suprarenal secretion, viz., that the blood pressure is so reduced after suprarenal removal that the blood stagnates in the relaxed auricles and clots there very slowly, giving rise to a tenacious mass of fibrin.

In conclusion, we desire to thank Professor C. J. Bartlett, of this School, for his kindness in making histological examinations of the clots, and giving us the benefit of his opinion as to their age.

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[1] Since writing this paper we have obtained extensive ante-mortem clotting in a third animal which died thirty-four hours after removal of the second gland. In this animal, one clot was found in the superior vena cava and the two innominate veins. The clot was white colored and showed a constriction opposite a valve in one of the innominate veins. A second and more extensive clot was found which had its main mass in the right ventricle attached to the interventricular septum and blocking up completely the *conus arteriosus*. This clot also blocked up the pulmonary artery to beyond its bifurcation, sending a process into each branch of that vessel. Where the clot passed through the semilunar valve it was very much constricted, demonstrating its *ante mortem* formation. Another long process passed from the main mass up through the auriculo-ventricular opening. The clots were hard in texture and white in color, except at the ends of the processes where they were pink colored. There was a firm adhesion to the interventricular wall and *conus arteriosus*. The animal died from respiratory failure, the heart continuing to beat for four minutes after the last respiration.

ON THE ABSENCE OF THE ACTIVE PRINCIPLE AND CHROMOGEN OF THE SUPRARENAL GLAND IN THE HUMAN EMBRYO AND IN THE CHILD AT BIRTH.

BY B. MOORE AND C. O. PURINTON.

[*From the Physiological Laboratory of Yale Medical School.*]

IN the course of experiments upon the removal of the suprarenals in the cat an opportunity presented itself of testing the effects of removal in the new-born kitten.

It occurred to us that the comparatively large size of the suprarenal at birth, as stated in text-books of Human Anatomy, ought to furnish an easy means of removal, even in a small animal, from the operative point of view. It further seemed desirable in the case of a gland which is so large comparatively in the embryo and dwindles in early life, to attempt a study of the effects of removal in the young animal.

Accordingly, we undertook the removal of the suprarenals in two litters of kittens, one of which was three days old, and the other between four and five weeks. The suprarenals were successfully removed on one side in two kittens of each litter, and these experiments are still in progress, but in the operation we were surprised to find that the suprarenals were exceedingly small; in fact, not relatively any larger than in the adult cat. In the three days' kittens the suprarenals were exceedingly difficult to find and identify, and were not any larger than large pinheads, and in the month-old kittens probably measured about two millimetres across.

In consequence of this discovery we turned our attention next to an investigation of the suprarenals in the human embryo and new-born child.

The glands used in our experiments were taken from an embryo between four and five months old, and a child still-born at full term.

The size of the suprarenals at once confirmed the usual statement of anatomical text-books as to the relatively large size of the suprarenal in the human embryo and at birth, furthermore proving that the relative size compared to the kidney goes on increasing up to birth.

Thus in the embryo the suprarenals weighed 3.551 grams and the kidneys 8.492 grams, while in the child at full term the suprarenals weighed 9.572 grams and the kidneys 20.073 grams.

This disparity in the relative size of the suprarenal in the human embryo and child as compared with its size in the kitten suggested the idea that the function of the large embryonic human suprarenal might be different, and led us to test the effects of intravenous injection of extracts of the embryonic human suprarenal and also to make a chemical examination of the embryonic gland for the well-known suprarenal chromogen.

Accordingly, extracts of one in five of the suprarenal glands, both of the human embryo of four to five months and of the child at birth, were prepared and tested.

Both physiological and chemical tests gave a negative result. It was found that injections of one cubic centimetre of this one in five extract gave absolutely no rise in blood pressure in a dog in which one tenth of a cubic centimetre of an extract of one in one hundred of calf's suprarenal gave a maximal rise. Further, addition of dilute ferric chloride solution gave no trace of a coloration with the human suprarenal extract, while a deep green color was given by the one in one hundred extract of calf's suprarenal.

The conclusion must accordingly be drawn that in the embryonic condition and at birth the human suprarenal contains neither the chromogenic group nor the active principle which raises blood pressure on injection.

The absence of both is a further indication of the close connection of the chromogen and the active principle, although, as we have previously shown,[1] the activity of extracts of the glands can be removed without involving the destruction of the chromogen.

At the time these experiments were made we were unaware that any experiments had been made on the injection of embryonic suprarenal extract either human or animal, but since then our attention has been drawn to a recent publication by Švehla,[2] in which the effects of injection of extracts of embryonic human suprarenal and that of young children, as well as extracts from embryonic ox suprarenal, are described. This paper deals with the comparative effects of injection of extracts of embryonic thymus, thyroid, and

[1] MOORE AND PURINTON: Journal of physiology, 1897, xxi, 383; Proceedings of the American Physiological Society: This journal, 1900, iii, p. xvi.

[2] ŠVEHLA: Archiv f. exper. Pathol. u. Pharmakol., 1900, xliii, p. 321.

suprarenal, and the author finds that all these, including suprarenal, are inactive in the human embryo.

With regard to the absence of the active material of the suprarenal gland in the human embryo our results must therefore be regarded as an independent confirmation of those obtained by Švehla, who, however, does not apply any chemical tests for the chromogen.

In the embryonic ox Švehla obtains even at an early stage the typical effects of adult suprarenal extracts.[1] We have not yet been able to obtain embryonic suprarenal from animals; but if the suprarenal in the embryo ox is, as we have observed in the young kitten, of normal relative size compared to the adult, and not as in man of relatively large size, the difference in results of injection points probably to some interesting difference either in the manner or time of development.[2]

It is our intention to study the subject in man and animals from a histological point of view, and especially to attempt to find in what respects the medulla of the embryonic gland differs from that of the adult.

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[1] The embryonic sheep suprarenal also contains the active material, according to Langlois and Rehns. Quoted from Švehla, *loc. cit.*

[2] Since this paper was written we have removed the left suprarenal from a kid born in the laboratory. The gland was removed about three hours after the birth of the animal and was of relatively small size as in the kitten, or adult animals. An extract of the gland gave all the chromogen tests characteristically and on injection raised the blood pressure in the same manner as suprarenal extracts from adult animals.

ON THE TRANSFORMATION AND REGENERATION OF ORGANS.

By JACQUES LOEB.

[From the Hull Physiological Laboratory of the University of Chicago.]

I.

SEVERAL of the older scientists, for instance, Bonnet, Spallanzani, and Dalyell had occasionally observed that in the place of a head a tail may be regenerated in lower animals.[1] These casual observations had been considered as curiosities or pathological cases, and scientists took no further notice of them. It occurred to me that it might be possible to produce the substitution of one organ for another *at desire*, and that in this way we might gain an insight into the physiology of morphological processes. Having tried in vain to accomplish this result during the year 1888 in Kiel, I succeeded the following year at Naples. I found that if the foot of a Tubularian hydroid be cut off and the foot end of the stem surrounded on all sides by sea-water a head will be produced instead of a foot, while the same end produces a foot if it is in contact with some solid body, like the bottom of the aquarium. This arbitrary substitution of one organ by another I called heteromorphosis in contradistinction to the case of regeneration in which the same organ is reproduced. I succeeded in showing that phenomena of heteromorphosis can easily be produced in all kinds of hydroids and in Tunicates.[2]

Since then a great number of heteromorphoses in various classes of animals have been obtained. The most brilliant accomplishment in this field of science is undoubtedly Herbst's discovery that if in crustaceans the eye together with the optic ganglion be removed an antenna will be produced in the place of the eye, while if the eye alone is cut off an eye is regenerated. The presence or absence of the optic ganglion decides whether a regeneration or a heteromorphosis will follow.[3]

I found, very early in my experiments, that in certain hydroids a

[1] LOEB: Untersuchungen zur physiologischen Morphologie der Thiere. I. Heteromorphose. Würzburg, 1891.

[2] LOEB: *Loc. cit.*

[3] HERBST, C.: Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. Archiv für Entwickelungsmechanik, 1899, ix, p. 215.

heteromorphosis can be produced without any organ being cut off or any wound being inflicted upon the animal. In Antennularia — a hydroid common at Naples — the arrangement and orientation of the organs as well as the direction of growth is dominated by gravitation. The animal consists of a straight vertical stem, which forms stolons at its lower end and which carries small branches with limited growth at regular intervals. On the upper surface of these branches the polyps are found. If such a stem be suspended horizontally in the water the lateral branches which are directed downwards and which had finished growing, now begin to grow downwards very rapidly. At the same time the polyps on these branches disappear. The downward-growing parts no longer resemble the old side-branches but look like roots. A closer examination reveals the fact that they not only possess the morphological appearance of roots but also the physiological reactions of the latter, inasmuch as they are positively geotropic and stereotropic, while the branches do not show these forms of irritability. In this case the tissue of the polyps which disappeared seems to have been transformed into the tissue of roots.[1]

I made a similar observation shortly afterwards at Woods Hole in another hydroid, Margelis. When the *uninjured* points of a stem of Margelis are brought in contact with a solid body the point of the stem assumes the form and reactions of a root. It looks as if the contact with a solid body brought about a transformation of the stem into root material which is morphologically and physiologically different from the stem.[2] But as neither Antennularia nor Margelis is sufficiently transparent it was not possible to ascertain that a transformation of polyps and stems into stolons occurs in this case.

Miss Bickford made an observation in my laboratory which helped in making the assumption of a transformation of organs more probable. Small pieces were cut from a stem of a Tubularian hydroid. These pieces were smaller in size than a normal polyp. Miss Bickford found that within sixteen hours such a piece assumed the form of a polyp.[3] Driesch confirmed her observation.[4]

[1] LOEB: Untersuchungen zur physiologischen Morphologie der Thiere. II. Würzburg, 1892.

[2] LOEB: On some facts and principles of physiological morphology. Woods Hole biological lectures, 1893.

[3] BICKFORD, E.: Notes on regeneration and heteromorphosis of Tubularian hydroids. Journal of morphology, 1894, ix, p. 417.

[4] DRIESCH, H.: Zur Analyse der Reparationsbedingungen bei Tubularia. Vierteljahrschrift der Naturforscher-Gesellschaft. Zurich, 1896.

Last summer I had an opportunity to observe directly the trans-
formation of organs under the influence of contact.   My observations
were made at Woods Hole on a transparent hydroid, Campanularia.
This hydroid attaches itself with stolons to solid bodies.   The stem
with the polyps grows at right angles with the solid body to which its
stolons are attached.   If these Campanulariæ be cultivated on a ver-
tical wall all the stems assume an exactly horizontal position in the
water.   The stem of a Campanularia is the most perfect specimen for
negative stereotropism I have ever observed.   If a stem be cut off

Fig. I.
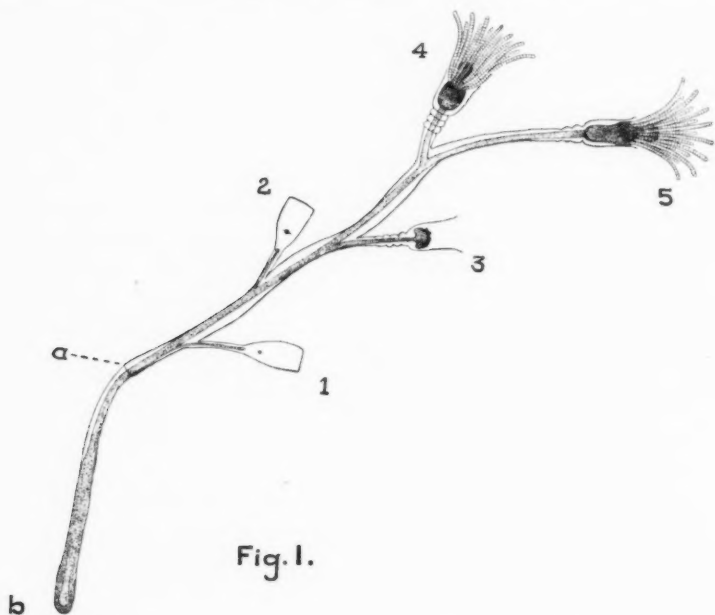
and put on the bottom of a watch glass filled with sea-water, all the
polyps that touch the glass are transformed into the material of the
stem.   This material creeps out of the stem, forming stolons wherever
it comes in contact with the glass, giving rise to polyps on its upper
surface which is in contact with sea-water.   The polyps continue
growing at right angles toward the bottom of the dish.   All these
processes may occur in less than a day, and can be observed directly
with a lens.   I will try to give a description of these phenomena with
the aid of camera drawings I made while observing them.   Fig. 1

shows the condition of a Campanularia stem that had been put on the bottom of a watch glass the previous day. Originally it had five perfectly developed polyps. Only two of these are left (4 and 5); the three others (1, 2, and 3) have disappeared. At the lower end, *a*, of the original stem a new stolon, *a b*, has grown out. What had become of the three polyps that had disappeared? I watched them very closely and found that they were transformed into a shapeless mass and withdrawn into the stem. I will describe this process of transformation of polyps into the material of the stem more minutely with the help of Figures 2, 3, and 4. These are not taken from the same stem, but as the process occurs almost always in the same form, this makes no material difference.
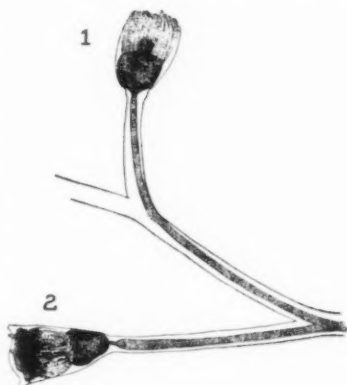
Fig. 2.

The transformation of a polyp into the less differentiated material of the stem begins with a shortening and folding together of the tentacles (polyp 1 in Fig. 2). This process is at the beginning the same as that which occurs upon any stimulation of the polyp and especially in the act of taking up food. But while in the latter case the tentacles unfold again, in the case of the transformation of the polyp they remain together. Very soon all the tentacles begin to fuse into a homogeneous mass. This process of fusing begins usually at the peripheral end of the polyp (polyp 2, Fig. 2). A little later all the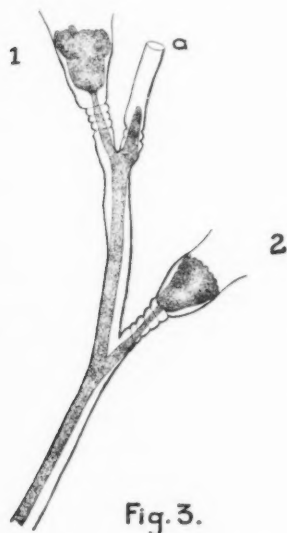 tentacles form an undifferentiated mass of protoplasm (see polyp 1, Fig. 3). In the next stage (2, Fig. 3) the original differentiation of the crown of the polyp into tentacles can no longer be recognized.

Fig. 3.

At this stage the transformed shapeless mass of the polyp begins to flow back into the stem (1, Fig. 4). A little later only a fraction of the original protoplasm of the polyp is left in the periderm, the rest having crept back into the stem (2, Fig. 4). In polyps 3, 2, and 1 (Fig. 1) we see the further stages of this process of the polyp material flowing back into the stem.
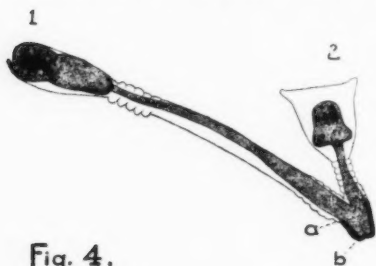
The transformation of polyps and their creeping into the stem occurs probably in a similar way in an Antennularia which is put into the water horizontally. The main difference between an Antennularia and a Campanularia is that in the latter this transformation is produced by the polyp coming in contact with a solid body, while, in an Antennularia a change in the position of the polyp towards the vertical suffices to bring about this result.

Fig. 4.

While these processes are going on, the material of the stem begins to creep or grow out of the original periderm. It seems to me worth while to call the attention of the reader to the fact that in this case *the process of growth is identical with the process of progressive motion of a protoplasmic mass.* In plants growth occurs mostly near the apex of an organ. If we look at the increase in size of the stolon from the point of view of growth we notice that its growing point is near the apex, just as in plants. But if we look at it from the point of view of progressive amœboid motion we notice that only the foremost point creeps and that the rest of the protoplasm is pulled out more passively. That the protoplasm of the stem is under a strain will be seen by a glance at Figs. 1, 2, 3, 4, and 5. The coenosarc or protoplasm lies in the periderm in the same way as a stretched rubber thread would lie. Wherever the periderm is bent the protoplasm touches it on the concave side. It follows as nearly as possible the *shortest line* in the periderm. It is possible that the strain under which the coenosarc is kept causes the protoplasm to flow in the direction of the strain towards the tip of the stolon. Botanists are inclined towards an exclusively osmotic conception of the process of growth. I have come more and more to the conclusion that the osmotic theory of growth is not in har-

mony with the phenomena of absorption. I do not consider it im-
possible that the phenomena of
protoplasmic motion which we
can actually observe in the growth
of a stolon in Campanularia exist
also in the phenomena of growth
of other organisms, plants as well
as animals. I have already called
attention to this possibility in a
former paper.

Before we leave this subject I
wish to describe how the nature
of the contact localizes the devel-
opment of polyps from stolons
and stems. The piece, *b c*, Fig. 5,
was cut out from a fresh Cam-
panularia stem and had been put
into a watch-glass filled with sea-
water. This piece had a normal
polyp at *i*, which was transformed
into a mass of undifferentiated
protoplasm and began to flow
back into the stem. Simultane-
ously a new stolon began to grow
out at *c*, and very soon reached
the considerable size, *c d*. Then
a new polyp, *h*, began to rise on
the upper surface of the stem.
It grew at right angles towards
the watch-glass, a point which in
the drawing cannot be rendered
accurately. A new stolon, *a b*,
began to grow or creep out simul-
taneously at *a*. Curiously enough,
as soon as this happened the pro-
toplasm began to flow back from
the old stolon, *c d*. At the time the
drawing was made it had flowed
back to the point *e*. This was on
the third day of the experiment. I have however noticed that the stem
can send out stolons in different directions simultaneously.
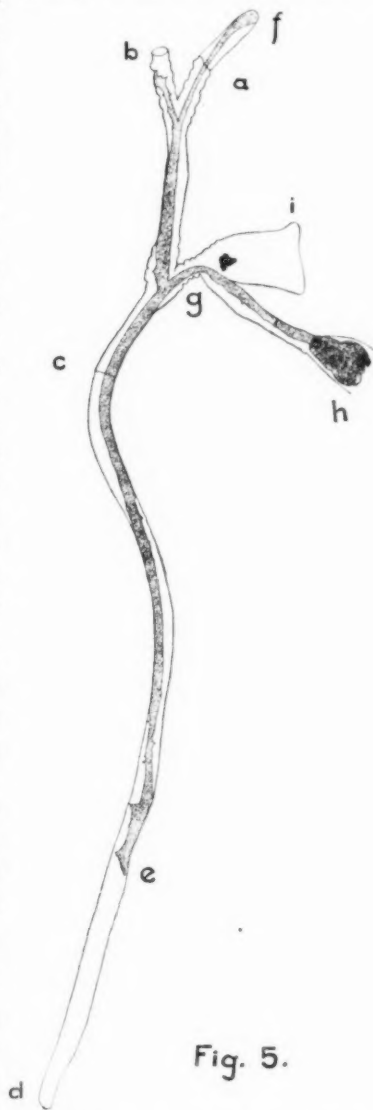

Fig. 5.

The hereditary arrangement of organs in hydroids is unequivocally determined by external circumstances, especially contact. A germ or larva of a hydroid will form roots on one side only, namely the side where it touches solid bodies: on the opposite side where it touches sea-water it will produce polyps or stems. The negative stereotropism of the latter or their positive heliotropism as in the case of Eudendrium will cause them to continue growing away from the solid body into the sea-water. Weismann is therefore wrong in assuming that the hereditary arrangement of the organs in hydroids is due to a definite arrangement of the elements in the germ.

II.

What is the character of the physical or physiological processes which underlie the transformation of organs? Such complicated formations as the polyp in Campanularia are only possible if certain of the constituents are solid. The transformation of such a polyp into the more shapeless flowing or creeping material of the stem can only be due to a liquefaction of these solid constituents. It is moreover certain that contact with sea-water favors the formation of polyps with its more solid elements while the contact with solid bodies favors the formation of the more fluid material of the stem or stolon. Hence it seems as if the nature of contact in this case determined the state of matter of certain colloids in the Campanularia.[1] Although I had observed the influence of the nature of contact upon these phenomena for many years I had not been able to form any definite idea of how the nature of the contact could possibly influence these processes, and I do not think that any one else has thus far offered an explanation. While studying the literature on the coagulation of the blood I came across Duclaux's account of this process in his Traité de Microbiologie,[2] and it seemed to me that if his notions are correct they might also be applied to our problem of contact-heteromorphosis. According to Duclaux it is the character of the contact applied to the leucocytes which decides whether the enzyme of coagulation, the plasmase, becomes effective or not. As long as the leucocyte touches the endothelium of the blood-vessels the blood remains liquid because the contact of the leucocytes with the endothelial cells does not allow the fibrin enzyme to act. If,

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[1] I do not need to mention especially that the periderm does not participate in these liquefactions.

[2] DUCLAUX: Traité de microbiologie, ii, Paris, 1899.

however, the leucocyte touches a piece of glass the plasmase becomes active and causes coagulation. If the glass is covered with a layer of oil coagulation does not occur. Duclaux assumes that surface tension phenomena decide the setting free of plasmase on the part of the leucocyte. Whether this latter assumption be correct or not matters little for our purpose. We only need to carry the analogy between the influence of contact upon the state of matter of fibrinogen and the state of matter of certain colloids in the hydroids far enough to assume that both depend upon definite enzymes becoming active through certain forms of contact acting upon the cells in which they are formed. In the case of the blood a solidifying enzyme, in the case of the polyps a liquefying enzyme is made active if the leucocyte or the polyp come in contact with glass or some other solid body.

These considerations possibly allow of a wider application than to the mere case of contact heteromorphosis. When a piece of our skin is cut off, the cells of the margin of the wound begin to multiply and spread out over the gap. We might say the change in the character of the contact causes an increase in the cell divisions. This is still more obvious where whole organs are produced or regenerated. In one of my former papers I pointed out a very definite chemical difference between embryonic tissue, and muscle tissue.[1] The former is more immune against K-ions and more sensitive towards Ca-ions. It has long been noticed, especially by botanists, that young tissue contains comparatively more K than old tissue. I am inclined to assume that this accounts for the fact that young tissue contains more water or has a greater degree of turgidity than older tissue. An increase in K allows the protoplasm to take up more water, an increase in Ca has the opposite effect.[2] Ion effects and the effects of certain enzymes of liquefaction or solidification are often similar, or may at least support each other. It is not impossible that the increase in cell divisions among the cells of the margin of the wound may be due to the different character of the contact to which these cells are exposed during or after the lesion, inasmuch as this different contact sets free or throws into activity certain enzymes which

[1] LOEB: On the different effect of ions upon myogenic and neurogenic rhythmical contractions and upon embryonic and muscular tissue. This journal, 1900, iii, p. 383.

[2] LOEB: Ueber die Aehnlichkeit der Flüssigkeitsresorption in Muskeln und in Seifen. Archiv f. d. ges. Physiol., 1899, lxxv, p. 303.

do not act as long as these cells are in their natural surroundings,
e. g. as long as they are in contact with other cells.

In returning after this digression to our main subject we must
mention that the nature of the contact is not the only means by which
solid elements in living tissues may be liquefied. Five years ago I
proved that lack of oxygen liquefies the cell walls in the blastomeres
of a teleost egg (Ctenolabrus)[1] and Budgett showed in my laboratory
that lack of oxygen produces the same phenomenon in Infusoria.[2]
This case may find its explanation through the well known experi-
ment of Pasteur on the effect of oxygen on yeast cells. With plenty
of oxygen the yeast cells multiply abundantly, but produce compara-
tively little fermentation; with little oxygen they multiply less but
cause a more abundant development of alcohol and CO_2. In the
liquefaction of the cell walls of the blastomeres of Ctenolabrus or of
Infusoria we may have the analogue of the increased fermentation in
Pasteur's experiment. In the latter we have to deal with a special
enzyme, the zymase.

Miescher pointed out that in the salmon a liquefaction of muscular
tissue occurs and that the liquid products are utilized for the forma-
tion of sexual cells. Miescher was inclined to ascribe the liquefaction
of the muscle to lack of oxygen. He noticed that the liquefaction of
the muscle was preceded by a reduction in the blood supply of the
muscles.[3] My own and Budgett's observations agree with Miescher's
views.

It is possible that the processes of histolysis in the metamorphosis
of insects are of a similar character, and some authors have claimed
that the histolysis in this case is brought about by a process of
asphyxiation. Metschnikoff assumes that phagocytosis plays an im-
portant rôle in these phenomena of histolysis. It is certain that in
my experiments on Ctenolabrus and in Budgett's experiments on In-
fusoria no phagocytes were present, and it is practically impossible
that they played a rôle in the above mentioned phenomena in Cam-
panularia. I do not think that the liquefaction of colloids requires
the presence of phagocytes any more than the liquefaction of crystals.

[1] LOEB: Ueber die physiologischen Wirkungen des Sauerstoffmangels. Archiv
f. d. ges. Physiol., 1895, lxii, p. 249.

[2] BUDGETT, S. P. On the similarity of structural changes produced by lack of
oxygen and certain poisons. This journal, 1898, i, p. 210.

[3] Die histochemischen und physiologischen Arbeiten von F. Miescher. Leipzig,
1897, i, pp. 94–100.

THE ELEMENTARY COMPOSITION AND HEAT OF COMBUSTION OF HUMAN FAT.

By FRANCIS GANO BENEDICT and EMIL OSTERBERG.

[From the Chemical Laboratory of Wesleyan University, Middletown, Connecticut.]

THE technical importance of the fats and oils has resulted in the extensive investigations regarding their composition which began with the exposition by Chevreul [1] in the early part of the century. The close analogy apparently existing between the different forms of those triglycerides collectively termed " fats " has led to the assumption that human fat has a composition not materially different from that of any other member of the series. Accordingly, the investigation of the elementary composition and the heat of combustion of human fat has received no special attention in recent years. However, with the advent of more accurate methods of studying the metabolism of matter and energy in the human body the necessity for more exact data regarding human fat is apparent.

Chevreul [2] reports human fat as having the composition 79.000 per cent carbon and 11.416 per cent hydrogen, but as Schulze and Reinecke [3] point out, these calculations were made using the value 76.53 as the atomic weight of carbon, while if calculated with our present standards these results become 77.85 per cent and 11.42 per cent respectively.

Heinz [4] in studying the glycerides present in fats from different sources concludes that human fat consists of a mixture of triolein, tripalmatin, and tristearin. No elementary analyses of the fats as such were made, though numerous analyses of the acids resulting from the saponification of the fat are given.

It is worthy of note that Lerch [5] a few years before proved the presence of volatile fatty acids, especially caprylic acid, in human

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[1] CHEVREUL: Recherches sur les corps gras d'origine animale. Paris, 1823.

[2] CHEVREUL: Loc. cit.

[3] SCHULZE and REINECKE: Liebig's Annalen der Chemie, 1867, cxlii, p. 191.

[4] HEINZ: Poggendorf's Annalen der Physik und Chemie, 1852, lxxxvii, p. 577.

[5] LERCH: Liebig's Annalen der Chemie, 1846, lix, p. 57.

fat. Traces of free fatty acids were found in human fat by Hofmann,[1] and this observation has also been made in recent years by Langer,[2] though instead of caprylic acid he found only butyric and caproic acids.

It is to the elaborate investigation of Schulze and Reinecke[3] that we are indebted for our knowledge of the elementary composition of human fat. After numerous analyses of fats from different animals, from different parts of the same animal, and from animals of different ages, the conclusion was reached that the animal fats have the general composition

$$C = 76.5\% \quad H = 12.0\% \quad O = 11.5\%$$

Human fat is included in this average. Two specimens of human fat were analysed and three analyses of each specimen are reported.

	Carbon. Per cent.	Hydrogen. Per cent.
Fat from kidney	76.38	11.90
	76.47	12.05
	76.46	11.88
Average	76.44	11.94
Fat from panniculus adiposus	76.80	11.95
	76.79	11.92
	76.81	11.97
Average	76.80	11.94

If these results are averaged the percentage composition of human fat is 76.62 per cent carbon and 11.94 per cent hydrogen.

In works on physiological chemistry the general average of all animal fats found by Schulze and Reinecke, *i. e.*, 76.5 per cent carbon, 12 per cent hydrogen, and 11.5 per cent oxygen, is ordinarily given as the composition of human fat.

The method of separating the fat from the membrane adopted by these writers is worthy of note. The fat, freed from all flesh and blood, was placed in a porcelain dish and dried in a steam bath from two to three days, and finally for twenty-four hours, or to constant weight, in an air-bath at $110-115°$. The melted fat was then filtered off and the membrane extracted with ether to remove all fat, which

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[1] HOFMANN: Cited in Physiological and pathological chemistry, Charles, p. 93.

[2] LANGER: Monatshefte für Chemie, etc., 1881, ii, p. 395.

[3] SCHULZE and REINECKE: *Loc. cit.*

was finally incorporated with that obtained by melting. The mixture was then again melted and filtered through filter paper. This treatment gave a clear, yellowish liquid, which when burned yielded no ash.

The methods of thermochemistry applicable to determining the heat of combustion of organic compounds are of comparatively recent introduction. The first combustions of human fat were made by Stohmann [1] in 1884 by a modification of the potassium chlorate method devised by Lewis Thompson.[2] Five determinations of the heat of combustion of human fat were made with two specimens. That from the panniculus adiposus gave 9.343, 9.401, and 9.394 large calories per gram, and that from the kidneys gave 9.445 and 9.409 large calories per gram. No description is given of the method of preparing the fat.

After the introduction of Berthelot's [3] bomb Stohmann [4] repeated the combustions of many of the substances he had previously burned by the potassium chlorate method and found that in general the former heats of combustion of the fats were 135 small calories per gram too low. The results of determinations of a number of specimens of fat from different animals agreed so closely with one another that he considered the heat of combustion of all animal fats (including human fat) to be 9.500 large calories per gram. No specimen of human fat has, so far as we know, ever been burned in the calorimetric bomb, and the only values available at present are those obtained by applying the correction of $+135$ calories suggested by Stohmann to the values obtained by the potassium chlorate method; *i. e.*, for fat from the panniculus adiposus 9.478, 9.536, and 9.529 large calories per gram, and for that from the kidney 9.580 and 9.544 large calories per gram. The average of these five corrected results is 9.533 large calories per gram. The heat of combustion of human fat is variously stated at from 9.300 to 9.500 large calories per gram.

The specimens of adipose tissue used in the work here reported, together with the description of the patients, were kindly furnished by Dr. A. R. Defendorf from autopsies at the Connecticut Hospital for the Insane in Middletown. In no case were any post-mortem changes observed. The specimens were obtained during the month of January.

[1] STOHMANN: Journal für praktische Chemie, n. F., 1884, xxxi, p. 275.
[2] Described by Frankland, Philosophical magazine, 1866 (4), xxxii, p. 182.
[3] BERTHELOT: Annales de chimie et de physique, 1888 (5), xxiii, p. 160.
[4] STOHMANN: Journal fur praktische Chemie, n. F., 1890, xlii, p. 361.

DESCRIPTION OF SPECIMENS.

1. From the panniculus adiposus abdominalis of a woman eighty-four years of age.   The patient was poorly nourished.

2. Perinephritic adipose tissue from the same patient.

3. From the panniculus adiposus abdominalis of a poorly nourished man seventy years of age.

4. Perinephritic adipose tissue from the same patient.

5. Perinephritic adipose tissue from a well nourished woman eighty years of age.

6. Perinephritic adipose tissue from an emaciated woman eighty-four years of age.

7. From the panniculus adiposus abdominalis of an emaciated woman fifty-one years of age.

8. Perinephritic adipose tissue from a poorly nourished woman ninety-two years of age.

SEPARATION AND PURIFICATION OF THE FATS.

The adipose tissue was placed in a beaker, heated gently in a water-bath for five minutes, and the melted fat poured through a perforated porcelain plate.   The residue was rubbed in a mortar and the operation repeated until the membrane was nearly freed from fat. The membrane was not extracted with ether.   The liquid fat containing particles of membrane was then filtered through a folded filter in a large water oven at a temperature of about 55 to 60 degrees. Filtration was complete as a rule at the end of twenty minutes.   The resulting fat when melted was clear, yellowish, and appeared not unlike olive oil.   No globules of water or particles of foreign material were to be observed.   The fat was then transferred to a large test tube, securely corked, and placed out of the light in a cool place.

While in the melted state the specimens presented no material differences in color or viscosity.   Certain specimens evidently contained more of the liquid triglyceride olein, than the others, as shown by the noticeable differences in the solidification of the specimens.   Specimens numbered one and two remained liquid a long time after melting and at no time completely solidified.   On standing, the solid triglycerides stearin and palmatin settled, leaving the liquid olein at the top.   Specimens three and four contained the greatest proportion of palmatin and stearin, as they both became completely solidi-

fied. The other specimens were all of about the same semi-liquid consistency at the room temperature.

In preparing and purifying these fats it was considered advisable if possible to avoid prolonged heating, especially in the presence of air. The oxidizing action of air on heated fats is a serious source of error in making water determinations of vegetable products containing oils or fats, and as subsequent experiments showed air is not without appreciable action on human fat. Inasmuch as the fats were not subjected to any especial drying operation during their preparation, it was necessary to prove the absence of water. When determinations of moisture were made by heating a few grams of the fat in a glass dish with or without the use of sand to increase the surface, a not inappreciable oxidation was obtained, the increase in weight amounting at times to over one third of one per cent. The drying operation when conducted in a current of hydrogen showed the absence of moisture.

ANALYTICAL RESULTS.

The determinations of carbon and hydrogen were made according to the modification of the method of Liebig described by one of us.[1] The determinations of the heat of combustion were made with a modification of Berthelot's bomb calorimeter described by Atwater and Blakeslee.[2] The apparatus as well as the manipulations were frequently tested by combustions of material of known purity and with a known heat of combustion.

In taking a sample for analysis the test tube containing the fat was immersed in water at 60 degrees till the fat was entirely melted. A slow stream of air freed from moisture and carbon dioxide was gently blown through the liquid to effect thorough mixing. The liquid fat was then drawn up into a cubic centimetre pipette and allowed to drop into the previously weighed porcelain boat used in the combustion tube or the platinum capsule used in the bomb calorimeter.

The carbon and hydrogen combustions presented no especial difficulty. In general they were completed in one and one fourth hours.

Table I shows the results of these combustions, each sample being determined in triplicate.

[1] BENEDICT: American chemical journal, 1900, xxiii, p. 335.

[2] ATWATER and BLAKESLEE: Tenth annual report Storrs (Conn.) Agricultural Experiment Station, 1897. p. 199.

The greatest differences in the percentage of carbon are noted in samples two and three, amounting, however, but to 0.51 per cent, or

TABLE I.

Composition of human fat.

Sample number.	Weight taken. Gram.	Weight water found. Gram.	Weight carbon dioxide found. Gram.	Hydrogen. Per cent.	Carbon. Per cent.
1	0.1428	0.1510	0.3994	11.83	76.28
	0.1211	0.1269	0.3382	11.73	76.17
	0.1194	0.1266	0.3346	11.86	76.43
Average	....	....	....	11.80	76.29
2	0.1208	0.1262	0.3386	11.68	76.44
	0.1177	0.1224	0.3292	11.64	76.28
	0.1205	0.1274	0.3374	11.83	76.36
Average	....	....	....	11.72	76.36
3	0.1420	0.1515	0.3949	11.94	75.84
	0.1442	0.1529	0.4008	11.86	75.80
	0.1502	0.1586	0.4180	11.82	75.91
Average	....	....	....	11.87	75.85
4	0.1526	0.1617	0.4246	11.86	75.89
	0.1540	0.1630	0.4289	11.84	75.96
	0.1268	0.1244	0.3534	11.86	76.01
Average	....	....	....	11.85	75.95
5	0.2070	0.2171	0.5762	11.74	75.92
	0.1442	0.1504	0.4017	11.67	75.97
	0.1235	0.1305	0.3439	11.82	75.94
Average	....	....	....	11.74	75.94
6	0.1136	0.1168	0.3170	11.51	76.10
	0.1415	0.1479	0.3943	11.70	76.00
	0.1282	0.1358	0.3578	11.85	76.12
Average	....	....	....	11.69	76.07
7	0.1216	0.1283	0.3394	11.81	76.12
	0.1234	0.1307	0.3440	11.85	76.03
	0.1228	0.1300	0.3433	11.84	76.24
Average	....	....	....	11.84	76.13
8	0.1205	0.1266	0.3352	11.76	75.87
	0.1401	0.1477	0.3910	11.80	76.11
	0.1365	0.1446	0.3812	11.86	76.17
Average	....	....	....	11.81	76.05
Average of 8 samples (24 determinations)				11.78	76.08

less than 0.8 per cent of the total carbon present. In the light of the striking agreement between the samples as a whole, the average

76.08 per cent for carbon and 11.78 per cent for hydrogen cannot be far from correct, and probably would be very little if any affected by a multiplication of samples.

The variations in the percentage of hydrogen are likewise inconsiderable.

In determining the heat of combustion the usual method of ignition by means of a small crystal of naphthaline was employed. The heat of combustion of the naphthaline, iron (also used in ignition), and the nitrogen oxidized to nitric acid were all deducted from the heat as measured. No difficulties were experienced in burning these samples.

Table II shows the results of these determinations.

TABLE II.

Heat of combustion of human fat at constant volume.

Sample number.	Heat of combustion per gram. Calories.	Sample number.	Heat of combustion per gram. Calories.
1	9.469 9.488 9.466	5	9.584 9.546 9.552
Average	9.474	Average	9.561
2	9.493 9.510 9.506	6	9.505 9.489 9.520
Average	9.503	Average	9.505
3	9.509 9.524 9.518	7	9.551 9.583 9.573
Average	9.517	Average	9.569
4	9.519 9.492 9.527	8	9.548 9.529 9.544
Average	9.513	Average	9.540
Average of 8 samples (24 determinations)	9.523 calories per gram.		

The agreement between the different samples is reasonably close, the existing variations not warranting any conclusion as to any material difference in the heat of combustion of the different samples. Sample one is somewhat lower and samples five and seven consider-

ably higher than the average, but at least four out of the eight samples, *i. e.* numbers two, three, four, and six, representing fat from three different individuals, give results practically identical with each other and with the general average.

The results given in Table II are those obtained for constant volume, and should be corrected [1] for constant pressure by adding 15 small calories to the heat of combustion per gram. Therefore the average heat of combustion of one gram of human fat at constant pressure is $9.523 + 0.015 = 9.538$ large calories.

In considering these average values the question doubtless should be raised whether specimens of fat from persons as aged as the greater number of these patients were, would represent fairly the composition and heat of combustion of average human fat. While all were over fifty years of age, there is a difference between the maximum and the minimum of forty-one years in the case of specimens seven and eight. The variation both in analysis and heat of combustion of these two samples is not much if any greater than the experimental error, and accordingly from the data given no difference in composition could properly be ascribed.

That any material difference in the composition of human fat from persons of different ages exists, seems not to have been considered until the appearance of the paper by Langer [2] in 1881. According to his researches on the fatty acids from two specimens of human fat, there was a considerable variation in the proportion of olein, stearin, and palmatin. The specimens examined were from a new-born child and a full-grown person. The conclusion is reached that in the fat of a new-born child a relatively small proportion of olein is present, and this is further shown by the fact that the fat has a much higher melting point than that from a full-grown person. It is hardly to be considered, however, that any material difference in composition or heat of combustion would exist in specimens of fat from adults of different ages. This opinion, given essentially by Schulze and Reinecke, seems to be substantiated by the results here presented.

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[1] STOHMANN: Journal für praktische Chemie, 1890, n. F., xlii, p. 363.
[2] LANGER: Monatshefte für Chemie, etc., 1881, ii, p. 382.

COMPENSATORY MOTIONS IN FISHES.

By E. P. LYON.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE results of previous experiments [1] had led me to believe that compensatory motions of the eyes persist in vertebrates in which both optic and both auditory nerves have been cut. A part of last summer, therefore, was spent in the study of the behavior of smooth dog-fish (*Mustelus canis*) on which that operation had been performed.

METHOD OF OPERATING.

In my former article I described a method of cutting the optic nerves of the dog-fish from the mouth. Further experience proves the plan perfectly feasible, and only a little more difficult, for the eighth nerves as well. The animal is placed on its back on a "shark-board" and fastened down with netting (a piece of old seine). The board is narrow in front, being about the width of the animal's head. A piece of cord fastened to one side of the board, then drawn tightly across the snout of the animal in front of the mouth and secured to the outer edge of the board, helps much to keep the dog-fish in proper position. The mouth is held open by placing a wire hook over the lower jaw and fastening it back to the netting. Artificial respiration is effected by conducting a stream of water to the back part of the shark's mouth. Even with its mouth fastened wide open and lower jaw immovable, the animal soon sets up respiratory motions of the gills and connected parts. Thereupon it becomes quiet and can be left on the board for several hours, if necessary, without apparent diminution of vitality.

A keystone-shaped piece of the skin of the roof of the mouth is raised and the connective tissue beneath scraped away. The optic nerves are then visible through a thin layer of hyaline cartilage near the front of the mouth. Each can be cut by a single stroke of a sharp scalpel. To make sure of the auditory nerves it is necessary to cut away some of the cartilage farther back in the mouth. This

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[1] LYON: This journal, 1899, iii, p. 86.

is best done with a small gouge. It is not necessary, however, to cut through into the brain cavity nor to molest the internal ear. The auditory nerve coming into view through the transparent cartilage may be cut by means of a curved knife. By inserting the knife into the septum between the brain and ear and cutting the nerve in its canal, all injury to the cranial cavity is avoided. The wound is dressed and the skin sewed up. This operation, which will be recognized as a modification of Schrader's method with frogs, can be performed without the shedding of a drop of blood, with all the nerves in plain sight, and without injury to the brain. It may be recommended to any who may operate upon the ears of dog-fish. Many of the animals lived in a small aquarium three or four days after the operation. This is about as long as normal animals will live in such a confined place. Post-mortem examinations of operated animals were made.

COMPENSATORY MOTIONS IN OPERATED ANIMALS.

After the operation the dog-fish were usually put back into the aquarium for a short time and then tested for compensatory motions by being rotated about the various body axes. From time to time other trials were made as long as the fish lived. I am able to say confidently that I had no animal whose optic and auditory nerves had been carefully cut that did not show some compensatory motion. On the other hand no animal showed as much or as regular and machine-like compensation as in the normal state. Usually the responses were much less than normal. The results, with one exception, to be mentioned later, were not constant in all individuals. One would perhaps manifest compensatory motions when turned toward the right about a dorsi-ventral axis. Another would respond on being rotated head downward about a transverse axis. In general it may be said that compensation on rotation in vertical planes was done away with in the majority of operated individuals. In all cases except one, the responses in these planes were inconstant, slow, and uncertain. One operated dog-fish compensated constantly and immediately every rotation about a transverse axis (head up or down), outside as well as in the water. The eyes did not, however, rotate in the socket opposite to the rotation of the animal, as do those of a normal fish. When the fish was turned head down, they simply rolled forward in the orbit so that the white was more visible

at the posterior end. When the fish was turned so that the head pointed up, the eyes changed so that the white was more visible in front.

Examination of normal individuals shows that, on rotation about a transverse axis, the eyes not only rotate about their own principal axes (this being the reaction which has been described by various observers), but also move forward or back in the orbits. On tipping the head of a normal dog-fish down, the white of the eyes is more visible in front, that is, at the anterior end of the eyeball. Turning the head up, the white shows more behind. The operated dog-fish described above had lost one part of its normal response to vertical rotation and retained the other part in exactly opposite sense to a normal fish. In other planes the animal showed no compensatory motions. The inexplicable and contradictory behavior of this individual is a good example of the confused and unnatural compensatory motions seen in fish whose second and eighth nerves had been severed. It may be claimed that these apparent remnants of compensatory motions were mechanical effects, the action of gravity upon the now uncontrolled eyeball, etc. This seemed to us impossible. Such motions were seen only in fish that were very lively, never in fish whose eyes had ceased to react to touch. These operated fish, moreover, moved their eyes spontaneously and seemed to have as good muscular control of them as uninjured individuals.

On the other hand, the fact must not be lost sight of that while some kind of compensatory motions could always be detected, I was not able to find an individual which showed these motions practically unchanged after the above described operation.

A New Compensatory Motion.

In handling these animals in the small laboratory aquarium it was often necessary to bend their bodies. It occurred to me that bending the body might have some effect upon compensatory motions. I therefore held the head (and consequently the semi-circular canals which are supposed to be dynamically stimulated), and bent the tail to one side. The eyes turned as promptly as compass needles. The same day Mr. Garrey pointed out to me that a normal dog-fish lying bent and at rest on the bottom of the aquarium always held the two eyes differently. Upon the convex side of the animal, the white was more visible in front; on the concave side, behind. This was

always found in all the individuals observed, whether normal or with the optic and auditory nerves severed. We also performed numerous experiments to test this apparently new reflex. Usually the head was fixed motionless on the shark-board and artificial respiration established. If then the tail was bent toward the animal's right side, the right eye turned forward, the left eye backward. The eye on the concave side of the animal was held at the front of the orbit; the eye on the convex side, at the back of the orbit. These relations were maintained as long as the tail was kept bent to one side. The animal being held with its median plane vertical and the motions of the tail being in horizontal planes, no question of gravitation enters. The head remaining fixed, no change of visual field or stimulation of semi-circular canals can be called in to explain the phenomenon. If the bending took place only near the tail, little or no eye motion resulted. The maximum effect was produced by bending the fish's body in the region of the anterior dorsal fin. This, however, was far enough back so that no mechanical stimulation of head organs seemed possible. Furthermore, since these compensatory motions were always present in animals whose optic and auditory nerves had been severed, all question of their mediation through the "equilibrium organs" of the head is precluded. No motion or unusual position of other parts, *e.g.*, fins, was observed. Bending the fins in various directions was tried without any response from the eyes. Bending of the tail up or down failed to produce corresponding motions of the eyes. We also tried the scup and other bony fishes. The head being held and the body bent laterally, every fish examined showed the same eye motions as the sharks.

To further test this reaction the spinal cord of several dog-fish was cut about two inches back of the medulla. After this operation, although the animals lived several days, the eyes showed no response to passive bending of the body. If the fish lay bent on the bottom of the aquarium, the eyes never appeared rotated in the direction opposite to the bending of the body. It would, therefore, seem that we had to do with a true reflex for which the cord is the afferent path.

It has been suggested [1] that the lateral line nerves are concerned in equilibrium. Stimulation of these nerves has caused eye motions very like those caused by stimulation of parts of the internal ear.

[1] LEE: This journal, 1898, i, p. 128.

We therefore thought, at first, that perhaps the lateral line organs had something to do with the motions of the dog-fish's eyes accompanying the bending of the body. But inasmuch as these nerves arise from the medulla, the failure of the reaction after the spinal cord was cut proved that this conjecture could not be true.

It would seem likely, therefore, that afferent nerves ending either in the skin or muscles, and which are consequently in a position to be stimulated by bending the body, constitute the afferent path. Through these nerves and by way of the spinal cord differences in the tension of the skin or muscles of the two sides of the animal cause impulses to be sent to the brain. These arouse efferent impulses which bring about the motion of the eyes.

The eye motion which accompanies the bending of the body is the same as that which occurs when the body is held straight and rotated in the same direction as the previous bending. In the latter case the compensating positions of the eyes are retained only during rotation, — imperfectly retained, however, since rapidly repeated nystagmus keeps bringing the eyes back to or toward their natural position. When the body is bent, the eyes maintain their compensating positions as long as the bent condition continues and no nystagmus is observed. Indeed this new compensatory motion is more like those previously observed on rotation of animals in vertical planes. In such cases the compensating positions are retained as long as the animal is held in unusual relations to gravity. In the new reaction the relations of the animal's own organs are changed, and the resulting compensation positions of the eyes are retained as long as these altered relations of organs continue. Shall we say we have to do with " static " equilibrium? At least we cannot make use of a " statocyst " or " statolith."

It may be asked whether this new source of compensatory motion does not account for all motions of the eyes seen after cutting the second and eighth nerves. I have no doubt that some of our early results were due to unwitting bending of the fish's body. Later, however, fish so operated were fastened perfectly straight and rotated. Traces of compensation could in some cases still be detected.

Compensatory motions independent of visual impressions and equilibrium sense organs of the inner ear do exist. If they have no other bearing, they show at least how careful we must be in building any theory of equilibrium organs upon observations of such motions.

The eyes are wonderfully responsive to change in the position of the body and to change in the relation of its parts. They are affected in so many ways that one can hardly be certain in a given case that he has eliminated all but one possible source of these motions.

My thanks are due to Dr. Loeb and Mr. Garrey for much assistance in observing the behavior of operated animals.

SUMMARY.

1. Passive bending of the tail of sharks and other fishes, the head being held at rest, causes compensatory motions of the eyes. The compensatory positions are retained as long as the body is kept bent.

2. Independent of the above reaction, dog-fish whose second and eighth cranial nerves had been severed showed traces of compensatory motions of the eyes. These motions are therefore unsafe guides in the study of equilibrium.

THE OCCURRENCE AND ORIGIN OF THE XANTHINE BASES IN THE FÆCES.

By WILLIAM H. PARKER.

[Instructor in Chemistry in the Yale Medical School.]

THE urine has been regarded, until quite recently, as the only excretory channel for the alloxuric bases. Weintraud[1] in 1895, however, observed a case of leukæmia in which there was only a small increase of uric acid in the urine, although an enormous increase of leucocytes was to be seen. This fact led him to examine the fæces for xanthine bases, and he obtained the remarkable result that the fæces of this leukæmic patient contained about ten times more of the xanthine bases than the urine. Indeed, the xanthine bases of the fæces, when precipitated by ammoniacal silver nitrate, gave an amount of nitrogen equal to about one gram of hypoxanthine per day.

An investigation of the fæces of healthy individuals further showed that these bases were constantly excreted through this channel, although in smaller quantities than under pathological conditions. Hence it must be admitted that the xanthine bases are normal constituents of the fæces, either as such or as components of contained nucleins.

Weintraud next examined the fæces after feeding large amounts of calves' thymus, which has been shown to be rich in nucleins, in order to determine whether the xanthine bases, present in the fæces, are directly derived from the food. In a healthy man, although large amounts of nucleins were ingested, there was no increase in the excretion of xanthine bases in the fæces, although a large increase of uric acid was observed in the urine. Further, on a purely milk diet, the xanthine bases obtainable from the fæces were undiminished in amount.

The results of these experiments indicate that the xanthine bases do not originate entirely from the food ingested.

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[1] WEINTRAUD: Centralblatt für innere Medicin, 1895, p. 433.

By quantitative experiments, using the Krüger-Wulff copper method, Weintraud [1] found in the fæces a daily excretion of xanthine bases of from 100 to 130 milligrams, and in his latest publication he [2] states that the amount of xanthine bases in the fæces daily varies between 100 and 500 milligrams.

The unreliability of the values obtained by the Krüger-Wulff method led Petrén,[3] in 1898, to repeat Weintraud's work using the Salkowski method. The fæces in Petrén's experiments were boiled with a two per cent solution of sulphuric acid in an open flask, the solution was then neutralized with barium hydrate, and slightly re-acidulated with sulphuric acid, to insure complete removal of the barium.

The filtrate, made slightly alkaline with ammonia, was allowed to stand several hours to precipitate ammonium magnesium phosphate, and the xanthine bases were then thrown down from the filtrate by ammoniacal silver nitrate. The values obtained by Petrén are much smaller than those published by Weintraud; they are further discordant in showing a variation of from 10 to 20 per cent.

In a healthy individual on a mixed diet, with a yield of twenty grams of dried fæces as the daily amount, there was an average excretion in the fæces of 68 milligrams of xanthine bases. Uric acid was never found, although the fæces were extracted with sulphuric acid, water, and sodium hydrate. If the daily excretion of xanthine bases in the urine be taken as 29 milligrams, a value which Flatow and Reitzenstein [4] found as an average of many analyses, using the Salkowski method, it will be readily seen from the analyses of Petrén that the amount of xanthine bases in the fæces is nearly twice as great as in the urine.

As this relatively large amount of xanthine bases in the fæces may have some clinical importance, it seemed desirable to continue and extend the investigations of Weintraud and Petrén, with a view to broadening our knowledge concerning the origin and significance of these bodies in the fæces.

Preliminary examination of human fæces showed that abundant

[1] WEINTRAUD: Wiener klinische Rundschau, 1896, 3; quoted from Maly's Jahresbericht.

[2] WEINTRAUD: Verhandlungen des 14ten Congresses für innere Medicin, 1896, p. 190.

[3] PETRÉN: Skandinavisches Archiv für Physiologie, 1898, viii, p. 315.

[4] FLATOW and REITZENSTEIN: Deutsche medicinische Wochenschrift, 1897, p. 354.

crystals of hypoxanthine silver nitrate could readily be obtained by use of the usual method, while xanthine could likewise be separated and its identity verified.

The plan of experimentation adopted was to study the influence of various diets, such as a carbohydrate, milk and bread, lean meat and Liebig's Extract, and calves' thymus, on a healthy man of average body weight; and to determine the amount of xanthine bases excreted in the fæces under each of these diets.

The fæces were divided into daily portions by the ingestion of a teaspoonful of animal charcoal. The fæces were weighed immediately after defecation, and then dried by evaporation on the water-bath with alcohol, as advocated by Pola.[1] The dried fæces were then ground fine, extracted with two per cent sulphuric acid on a boiling water-bath, the extract neutralized with barium hydrate, filtered, and the xanthine bases in the filtrate precipitated by ammoniacal silver nitrate.

The xanthine bases were then determined according to the Salkowski method for the determination and separation of uric acid and the alloxuric bases in the urine. Salkowski's factor, 1 Ag equals 0.7381 xanthine bases, was used in the calculation of the amount of xanthine bases throughout these experiments.

The xanthine bases excreted in the fæces on a carbohydrate diet. — A diet of 500 grams of rice and 50 grams of cane sugar, per diem, was ingested for a period of three days, with the results as to the excretion of xanthine bases shown in the following table.

Rice Period.

	Body weight in Kilos.	Fæces weighed wet in Grams.	Fæces weighed dry in Grams.	Xanthine Bases in Milligrams.	Total Nitrogen in Grams.
1st day	69.5	106.6	31.5	74.0	3.13
2d day	69.3	136.9	33.6	71.0	2.78
3d day	69.0	75.3	23.0	31.0	0.70

These results show a slight decrease in the excretion of xanthine bases on the second day, while on the third day a distinct drop is seen.

[1] POLA: Archiv f. d. ges. Physiol., 1897, lxix, p. 680.

The result on the third day may perhaps be taken as representing the normal excretion of xanthine bases on a carbohydrate diet, while the higher values for the first two days may be due to the gradual clearing out, by the coarse rice diet, of the xanthine bases from the preceding mixed diet.

A milk and bread diet. — The milk diet was commenced immediately after the carbohydrate diet of the preceding experiment, thus insuring complete freedom from the influence of the previous mixed diet.

It is well known that caseinogen does not yield xanthine bases on decomposition and, therefore, any excretion of these bases must be due to some other cause than the food ingested. The daily diet consisted of one quart of milk, 500 grams of bread, and 100 grams of butter. The results obtained are shown in the following table.

Milk Period.

	Body weight in Kilos.	Fæces weighed wet in Grams.	Fæces weighed dry in Grams.	Xanthine Bases in Milligrams.	Total Nitrogen in Grams.
1st day	68.7	77.5	23.0	30.0	0.79
2d day	68.8	122.4	25.5	28.0	0.90
3d day	69.1	124.0	27.7	38.0	1.02

The results of this period indicate that after complete cleaning out of the intestinal canal by a carbohydrate diet, the excretion of xanthine bases in the fæces on a milk and bread diet is fairly constant. The above figures therefore may be taken as representing the normal excretion of the xanthine bases and probably derived chiefly from the nuclei of the dislodged cells from the intestine.

The average daily excretion is 35 milligrams. The average daily excretion of xanthine bases in the urine during the same period was 23 milligrams, and this amount may perhaps be regarded as the normal amount of xanthine bases derived from the body tissues, and excreted by way of the kidneys. The average daily excretion of uric acid in the urine during this period was 0.681 grams: the average total nitrogen excreted through the urine was 14.6 grams.

A diet of lean meat and Liebig's Extract. — This period was commenced immediately after the milk and bread period.

Seven hundred grams of beef, weighed raw, and twenty-six grams of Liebig's Extract were ingested on the first day. As this diet seemed excessive, the amount of meat was cut down to 500 grams but no change was made in the amount of Liebig's Extract.

Meat Period.

	Body weight in Kilos.	Fæces weighed wet in Grams.	Fæces weighed dry in Grams.	Xanthine Bases in Milligrams.	Total Nitrogen in Grams.
1st day	69.6	52.0	21.2	62.0	1.07
2d day	68.6	98.2	23.2	66.0	1.80
3d day	68.5	136.6	34.5	81.0	2.36

This experiment shows an immediate increase in the excretion of xanthine bases under the influence of the meat diet plus Liebig's Extract. The average daily excretion was raised to 69 milligrams. This fact suggests that either there is a tendency in the human organism for a certain proportion of the xanthine bases of the food to escape from the body through the fæces as well as through the urine, or, as is perhaps more probable, that the above diet so stimulates the activity of the intestinal epithelium, etc., as to increase the output of nuclein material through the fæces. The appearance of the fæces, on the last day, indicated that some of the meat had not been completely digested. No free xanthine bases, however, could be found in the fæces.

This excretion is also practically double the amount obtained during the milk period.

The average daily excretion of uric acid and total nitrogen in the urine was 0.9876 and 20.50 grams respectively.

As my condition did not warrant undertaking further dieting at this time, a brief rest was taken before commencing the diet of calves' thymus. Preliminary to this period, opportunity was taken to make an observation on the amount of xanthine bases excreted in the fæces on a liberal mixed diet.

A mixed diet. — The mixed diet consisted of meat, potatoes, bread and butter in such quantities as desired. No coffee or tea was taken.

The xanthine bases excreted in the fæces during this period amount to a daily average of 57 milligrams.

Mixed Diet Period.

	Body weight in Kilos.	Fæces weighed wet in Grams.	Fæces weighed dry in Grams.	Xanthine Bases in Milligrams.	Total Nitrogen in Grams.
1st day	67.5	78.9	19.7	49.0	1.27
2d day	67.0	111.3	24.0	65.0	1.60
3d day	66.5	100.4	27.3	58.0	1.90

A diet of calves' thymus. — Five hundred grams of fresh calves' thymus were ingested per day. No other food was taken.

Nuclein Period.

	Body weight in Kilos.	Fæces weighed wet in Grams.	Fæces weighed dry in Grams.	Xanthine Bases in Milligrams.	Total Nitrogen in Grams.
1st day	66.5	66.7	30.1	59.0	2.15
2d day	66.4	117.9	30.3	73.0	2.10
3d day	67.1	113.1	36.8	76.0	2.81

This table shows that when nucleins are ingested in large amounts there is a slight rise in the proportion of xanthine bases in the fæces. The results of the last two days of the period show an excretion of almost 75 milligrams per day through the fæces, while the average excretion on a mixed diet was 57 milligrams and on a meat diet 69 milligrams.

Schindler[1] has shown that calves' thymus contains or yields about 227 milligrams of xanthine bases for every 100 grams of fresh tissue. Taking this figure as a basis, there was ingested then each day an amount of thymus sufficient to yield about 1.134 grams of xanthine bases. If we subtract the amount of xanthine bases excreted in the fæces during the milk period, which may be regarded as the normal intestinal excretion, there would be left an increase of only 40 milligrams after the ingestion of large amounts of true nucleins.

[1] SCHINDLER : Zeitschrift für physiologische Chemie. 1889, xiii, p. 438.

The average daily excretion of xanthine bases in the urine during the thymus period was only 51 milligrams. This shows that the xanthine bases in calves' thymus are changed to other bodies in metabolism and that only small amounts of these bases are excreted in the urine or fæces.

The average daily excretion of uric acid and total nitrogen in the urine during this period was 1.786 grams and 16.27 grams respectively. Uric acid was never found in the fæces during any of the periods of experiments.

An attempt was next made to ascertain whether free xanthine bases are present in the fæces. This was accomplished by boiling fresh fæces for several hours with water, and testing the filtrate for xanthine bases by the addition of ammoniacal silver nitrate. Negative results were invariably obtained, thus indicating that on a mixed diet the xanthine bases obtainable from the fæces must have their origin in some one or more nuclein compounds, presumably contained in the cell nuclei thrown off from the intestinal wall.

CONCLUSIONS.

From these experiments the general conclusion may clearly be drawn that under normal conditions on a diet containing no nucleins, there is always a constant excretion of combined xanthine bases derived from the cells of the alimentary canal. This excretion is about 30 milligrams per day, and is about equal to the excretion of the xanthine bases in the urine under similar conditions.

The xanthine bases are increased to nearly double the above amount when either a mixed diet, a diet of meat alone, or a diet of calves' thymus is consumed, showing that a large increase may arise from the nature of the ingested food, viz., nuclei containing food or food rich in alloxuric bodies, as meat and meat extracts. This increase, however, is not necessarily due directly to the ingested food, but may arise indirectly from an influence exerted on the processes of metabolism and secretion.

I wish to acknowledge the kind advice of Professor R. H. Chittenden, at whose suggestion these experiments were carried out.

PHYSIOLOGICAL STUDIES ON MUCINE.

By ISAAC LEVIN.

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IT has been established that the removal of the thyroid gland is very injurious; for many species of animals, as well as for man. it is fatal.

Various theories have been offered in explanation of this phenomenon. It may be due to the fact that some substance, which has either been previously transformed by the cells of the thyroid or else neutralized by some other substance produced by the gland, in the absence of the latter, accumulates in the blood and poisons the organism. But the nature of this toxic substance has never been established. The discovery of an increased amount of mucine in the tissues in myxœdema, as well as in thyroidectomized animals, led Horsley[1] and others to suppose a priori, that the symptoms of cachexia thyreopriva may be due to accumulation in the blood of mucine, which is normally transformed by the thyroid. In view of these possibilities, and since I was unable to find in the literature any physiological or pharmacological study of the influence of mucine on an organism, it seemed to me desirable to test the matter experimentally. In order to study the relation between mucinæmia and the thyroid, I availed myself of the fact, that rabbits endure thyroidectomy a great deal better than carnivorous animals. Until lately it was universally accepted that thyroidectomy was not fatal to rabbits. Gley[2] in his work endeavors to show that the operation is fatal to rabbits if all the parathyroids are also extirpated. But even with him only a small percentage develop an acute cachexia, and these die not later than within three days after the operation.

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[1] HORSLEY: Proceedings of the Royal Society of London, 1884, xxxviii. p. 5, and the British medical journal, Jan. 31, 1885, p. 211.

[2] GLEY: Archives de physiologie, 1892, p. 135, 1893, p. 467, and 1895, p. 771.

The rest either recover entirely, or emaciate and die a few months after the operation. I have obtained identical results in my simple thyroidectomies on rabbits. A few of the animals die within twenty-four or forty-eight hours, but by far the greater part survive. Taking this as a basis, I performed the following experiments. Into each of eight normal rabbits of between 1000 to 1200 grams in weight I injected hypodermically from $\frac{1}{2}$ to $\frac{3}{4}$ gram mucine in 1 per cent solution of sodium carbonate. The mucine used was prepared for me by Dr. P. A. Levene from fibrous connective tissues. All the rabbits remained perfectly healthy after the injection.

I then administered the same hypodermic injection to nine rabbits whose thyroids I had previously extirpated.

Here are the results:

Rabbit.	Weight in Grams.		Mucine injected.	Died.
N 1	1300		$\frac{3}{4}$ gram 1 day later.	3 days later.
N 2	1290		$\frac{1}{2}$ gram 14 days later.	5 days later.
N 3	1200		$\frac{3}{4}$ gram 22 days later.	42 hours later.
N 4	1240	Thyroidectomy.	$\frac{1}{4}$ gram 2 days later.	6 days later.
N 5	1320		$\frac{1}{4}$ gram 16 days later.	Alive.
N 6	1080		$\frac{1}{2}$ gram 2 days later.	6 days later.
N 7	1200		$\frac{1}{4}$ gram 25 days later.	2 days later.
N 8	960		$\frac{1}{2}$ gram 6 days later.	6 days later.
N 9	1210		$\frac{1}{2}$ gram 11 days later.	1 day later.

Thus out of nine rabbits eight died, a rate of mortality far higher than even Gley himself describes as the result of simple thyroidectomy. Moreover, some of the experiments, as Nos. 3, 7, and 9, where rabbits, after having survived the thyroidectomy for 11, 22, or 25 days respectively, died within twenty-four or forty-eight hours after the injection of mucine, are so striking, that there can hardly remain any doubt that mucine is toxic for an animal deprived of the thyroid.

I have also made some experiments on dogs, proceeding in the following way. Into a normal dog was injected intravenously a

certain quantity of mucine solution, which has never been found to
have any influence upon a normal organism. After several days the
thyroid was extirpated, and the bad effect of the operation was neu-
tralized by administration of iodothyrin. After the lapse of a few
more days the same animal was again injected intravenously with
the same amount of a mucine solution. All of the animals then
died, some of them in nine or ten hours after the injection. But
these experiments cannot be recorded, as not every dog can be kept
alive after thyroidectomy, even if iodothyrin is administered and
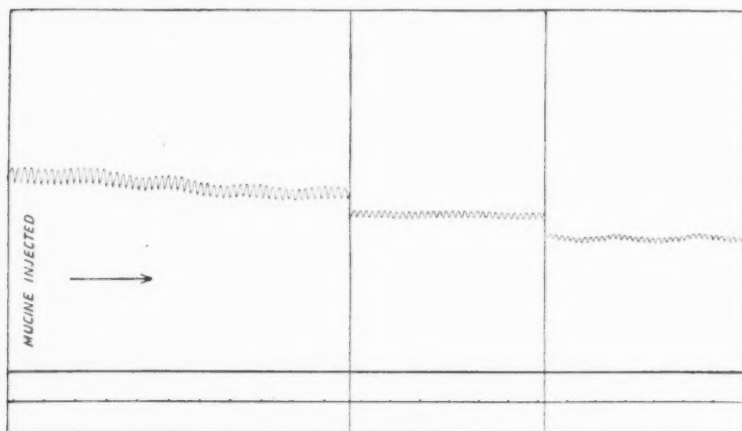mucine is not injected.

FIGURE 1. — Fall of the blood-pressure after an injection of mucine.
Read from left to right.

Now, if we are in a position to add to the established facts, that
mucine is one of the products of the proteid metabolism and that it
is found in increased quantities in the tissues of an organism deprived
of its thyroid, the new fact, that injection of mucine does not affect a
normal organism, and is fatal to a thyreoprived one, the supposition,
that cachexia thyreopriva is an autointoxication with mucine, is very
plausible indeed.

There seemed to me to be still another way to approach the
question experimentally. If mucinæmia is fatal for a thyreoprived
animal, it should also have at least some effect on a normal animal.
As we know that a number of the symptoms of cachexia thyreopriva
are of a nervous nature, it seemed that the most promising results

would be obtained from the study of the influence of mucine upon
the nervous system of a normal organism. One of the most exact
indicators of the influence of any factor on the nervous system is
the vasomotor centre as shown by the changes of the blood-pressure.
On five dogs I studied the influence on the blood-pressure of an
intravenous injection of a mucine solution. Into every one I injected
50 c.c. of 3 per cent solution of mucine in 1 per cent sodium car-
bonate. In every experiment, as seen on the tracings, the blood-
pressure was markedly decreased (Figs. 1 and 2). This decrease
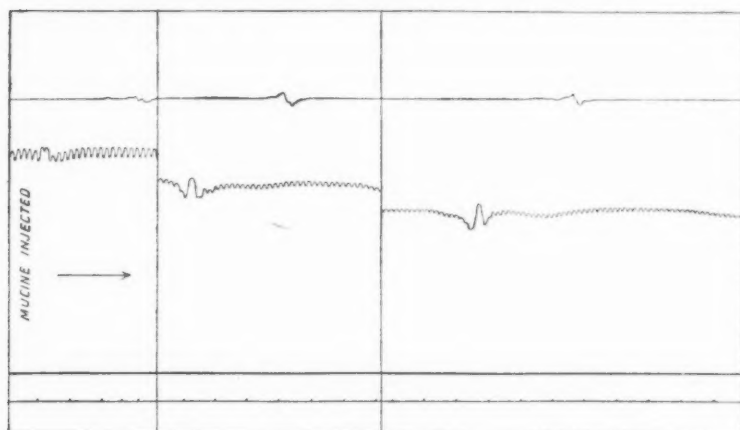was not due to the influence of mucine on the inhibitory mechanism

FIGURE 2. — Fall of the blood-pressure after an injection of mucine.
Read from left to right.

of the heart, since both vagi were cut in every experiment previous
to the injection. That it was also not due to the influence of mucine
on the peripheral vasomotor nerves I proved by the following ex-
periment: On a dog both vagi and the splanchnic were cut, and
then, when the blood-pressure reached its lowest point, mucine was
injected intravenously. The blood-pressure fell again. Every sub-
sequent stimulation of the peripheral end of the splanchnic increased
the blood-pressure. As soon as the stimulation ceased, the blood-
pressure fell again to the level reached by the mucine injection
(Fig. 3).

The decrease of blood-pressure after the intravenous injection of
mucine was due consequently to its direct influence upon the vaso-

Isaac Levin.
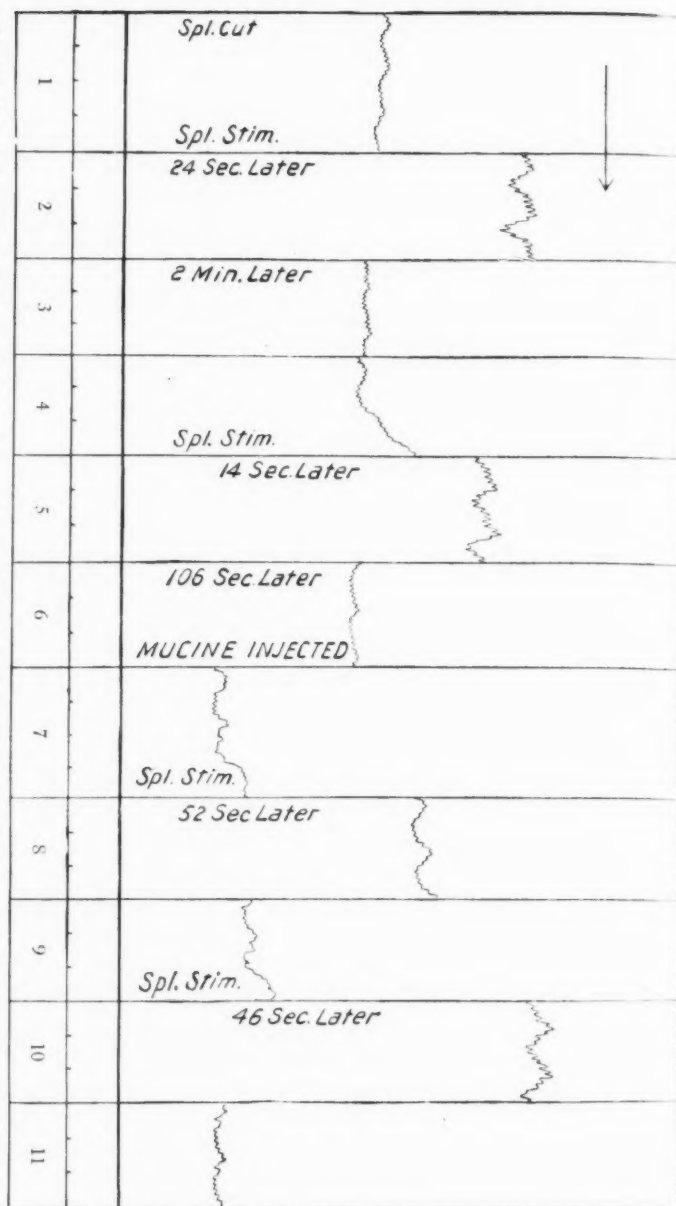
FIGURE 3.—Section of the splanchnic nerve with subsequent injection of mucine. Sections 1, 3, 4, and 6 show the height of the blood-pressure after the splanchnic has been cut. Sections 2 and 5 show the highest points the blood-pressure reached after the peripheral end of the splanchnic was stimulated. Sections 7, 9, and 11 show the fall of the blood-pressure after the injection of mucine. Sections 8 and 10 show the rise of the blood-pressure after the peripheral end of the splanchnic was stimulated, notwithstanding the presence of mucine in the blood. Read from left to right.

motor centres, which, in the presence of mucine in the blood, became depressed. It is interesting to note that the influence of mucine on the nervous system is of a depressive character, and of the same depressive character are the nervous symptoms in cachexia thyreo-priva (not tetanic).

Thus the conclusion I can draw from my work is that mucine accumulated or introduced into the blood of a normal organism produces a certain depressive effect upon the central nervous system; it is not fatal to a normal organism, but is decidedly fatal to an organism deprived of its thyroid. Mucinæmia then may be the pathological condition of an organism resulting from the absence of the thyroid function. But this conclusion does not exclude the possibility of other abnormalities arising from the same cause.

In conclusion I have the pleasant duty of expressing my gratitude to Professors T. M. Prudden, J. G. Curtis, and Frederic S. Lee, in whose laboratories the work has been done.

STUDIES ON ELECTROTAXIS. I.—ON THE REACTIONS OF CERTAIN INFUSORIA TO THE ELECTRIC CURRENT.[1]

By RAYMOND PEARL.

CONTENTS.

I. INTRODUCTION.

NEARLY all of the work on electrotaxis among the Protozoa has been done either to determine the sense of reaction, that is, whether anodic or cathodic, or to analyze the effect of the current on protoplasm. The only extended observations on the precise manner in which the organisms orient themselves with respect to the direction of the current are those of Ludloff,[2] who worked out very carefully the effect on Paramecium of different strengths of constant currents.

In comparing Ludloff's conclusions from a study of the electrical stimulus with those of Jennings,[3] who has more recently described the reactions of various Protozoa to stimuli other than electrical, it is seen that the method of reaction to the electric current does not at all agree with that in response to other stimuli. The "motor reaction"

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[1] Work from the Zoölogical Laboratory of the University of Michigan, Jacob Reighard, director.

[2] LUDLOFF: Untersuchungen über den Galvanotropismus. Archiv f. d. ges. Physiol., 1895, lix, pp. 525-554.

[3] JENNINGS: Studies on Reactions to Stimuli in Unicellular Organisms. V.— On the Movements and Motor Reflexes of the Flagellata and Ciliata. This journal, 1900, iii, pp. 229-260.

or " reflex " of the infusoria has in general terms been described by Jennings [1] as follows: " When unstimulated the animal swims with a certain structurally defined end (the " anterior ") in front. When stimulated, motion takes place with another (the " posterior ") structurally defined end in front, followed by turning towards one side which is structurally defined and invariable (the aboral in Paramecium), — finally succeeded by motion with the same end in front as at first." This general statement was reached at through a study of a great variety of chemical and physical stimuli acting on different Protozoa. The reaction to the current in Paramecium as described by Ludloff [2] is due to the increased effectiveness of the stroke of the cilia on the anode side, which causes the animal to turn till the anterior end is directed towards the cathode. It then swims off in that direction because the stroke of the cilia on the anode (posterior) half is stronger than on the cathode (anterior) half of the body. In this reaction there is no turning towards a " structurally defined " side, but simply towards the side which at the moment happens to be nearest the cathode. Evidently there occurs in Paramecium, in response to the electric current, a reaction different in kind from that in response to other stimuli, so far as the action of the latter is known.

In view of this fact it seemed desirable to work over Ludloff's results and extend them to other forms in order to discover, if possible, whether there is any relation between the two kinds of reaction. It is from such a standpoint that this investigation has been undertaken.

It may be well to put in the form of a definite question the exact problem which was set as a basis for this study: Does the reaction of the Protozoa to the electric current agree with their reactions to other stimuli, *e.g.* chemical, mechanical, etc., and how is the reaction to the current brought about?

The experiments will be taken up separately for each animal studied, and this descriptive part will be followed by a discussion of the relation of the results to the general problem of electrotaxis.

I wish to take here the opportunity to acknowledge my great indebtedness to Dr. H. S. Jennings, under whose direction and advice the work was done, and to express my sincere appreciation of his kindness and help to me.

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[1] JENNINGS: *Loc. cit.* pp. 229-230.
[2] LUDLOFF: *Loc. cit.*

II. Material and Methods.

The infusoria principally used in this study include one flagellate, Chilomonas paramecium Ehr., and the following ciliates: Paramecium caudatum Ehr., Colpidium colpoda Ehr., Spirostomum ambiguum Ehr., Stentor coeruleus Ehr., Oxytricha fallax Stein, and several other Hypotricha of undetermined species. These include among the Ciliata, as will be seen, representatives of the Holotricha, Heterotricha, and Hypotricha.

The current used was taken from the general lighting current supplied to the building at a voltage of 220 by a direct current dynamo. It was put through a 16 c. p., 220 volt incandescent lamp connected in series with a "Jewell Graphite Rheostat." From this rheostat a current, which could be varied from zero to about 350 M. A., was led off. In the circuit were a plain switch and a current reverser. The electrodes used were the non-polarizable brush electrodes described in various text-books of physiology.

In order to determine the exact method of reaction, it was necessary to have the animals in a very thin layer of fluid and this end was attained by supporting the cover glass of the preparation on strips of Japanese lens paper which were saturated with normal salt solution. On these strips the brushes of the electrodes rested, thus making the circuit complete. This method I used almost exclusively in the work. The animals were distributed in a very thin layer, the cross section of water through which the current was passing being about 1.2 sq. mm. The thickness of the layer of water was about .06 mm.

The intensity of the current is indicated throughout the paper by the terms "weak," "medium strength," and "strong." The estimated strengths of current designated by these terms are as follows: "weak" currents include all those of an intensity less than 8δ, "medium strength" includes all of intensities between 8δ and 20δ, and "strong" all above 20δ. Any finer distinction of current strength is not significant, because there is so much variation among individuals. What is evidently a current of "medium strength" to one may be "strong" to another.

III. Observations.

In describing the experimental results, I shall take up each Protozoön separately, arranging them according to their reactions. The reactions of Colpidium will be first described somewhat in detail to serve as a basis for comparison with the other forms.

COLPIDIUM COLPODA EHR.

When a current of medium strength is sent through a preparation containing a number of Colpidia it is seen that about half of them at once swim towards the cathode, while the remainder of the individ-

uals swim first at various angles across the direction of the current, but gradually are seen to become oriented and to swim towards the cathode, till finally nearly all the animals are swimming in that direction. The swimming at this current strength is a little more rapid than usual.

If now at the moment of making the current one particular Colpidium be observed, it will be seen

FIGURE 1. — Diagram to indicate the different positions of Colpidium with reference to the direction of the current.

that the orientation in the line of the current with the anterior end to the cathode depends on the position which the animal occupies with respect to the direction of the current.

If the Colpidium happens to lie in the direction of the current,

FIGURE 2. — Diagram showing Colpidium in orientation. *r*, "reflex cilia"; *p*, posterior end.

with the anterior end towards the cathode (Fig. 2), when the circuit is completed it swims for the cathode in a spiral path, or perhaps more exactly in a zigzag path (Fig. 5); that is, it starts forward at a small angle with the direction of the current, and after pursuing this path for a varying

distance, usually from six to ten times the length of the body, it pauses for an instant, rotates through 180° about the long axis, and, turning slightly towards the aboral side again, starts off on a new path, forming the second arm of the zigzag. This process is repeated
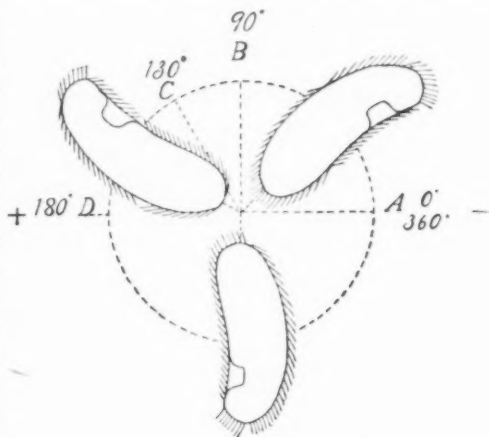
so that the path formed is made up of a series of equal angles with the animal swimming first on one side and then on the other. In case the animal is swimming towards the cathode when the current is made, it simply changes its path and keeps on.

If the animal is lying in the line of the current, with the anterior end towards the anode (Fig. 3), when the current is made, it immediately turns towards the aboral side through 180°, thus coming into orientation with the anterior end to the cathode, towards which it starts to swim in the manner just described. This method of reaction in which the animal simply turns to the aboral side in the ordinary motor reflex way, I have, for convenience, called Type I (Fig. 3).

If the animal happens to lie at the moment of making the current in such a position that the long axis forms *any angle* with the direction of the current, and the *aboral* side of the body is nearest the cathode (Fig. 1, positions D to A), then it reacts simply and quickly by whirling on the short axis of the body towards the *aboral* side till it comes into orientation with the anterior end to the cathode (Fig. 2), towards which it swims in the usual path. Evidently the reaction here is essentially the same thing as Type I, only beginning with the animal in a different position (Fig. 1, positions D to A) from that described for the typical case (Fig. 3).
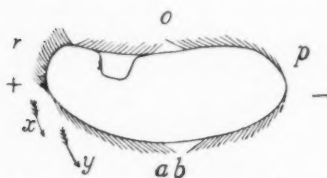
FIGURE 3. — Diagram showing Type I of reaction · *o*, oral side; *ab*, aboral side; *p*, posterior end; *r*, "reflex cilia"; *x*, arrow indicating the direction in which the reflex cilia tends to turn the animal; *y*, arrow indicating the direction in which the forced movements of the body cilia tend to turn the animal.

When, on the other hand, the Colpidium is lying at the moment of closing in such a position that the long axis of the body forms an angle with the direction of the current, and the *oral* side is nearest the cathode (Fig. 1, positions A to D), one of several reactions may take place, according to the size of the angle formed.

If the angle is less than 90°, reckoning from the cathode pole as zero (Fig. 1), the Colpidium, when the current is made, begins to swim slowly in a line slightly curved towards the oral side, that is, turning the anterior end slowly towards the cathode. It usually (in about 70 per cent of all 'cases) finally comes into orientation by directly turning towards the oral side in this way; but the process is a slow one, and the animal swims some distance at an angle to

the current direction before getting around. This method of reaction may be considered as another type, and I have designated it as Type II (Fig. 4).

Of the remaining 30 per cent, about one half become oriented in one way, and the other half in another. Of these two methods the first is as follows. The animal starts to turn towards the cathode as in the case just described, but when it has accomplished about half the necessary turn, it rotates on its *longitudinal* axis through 180°, and then quickly snaps around towards the aboral side into orientation, and swims to-
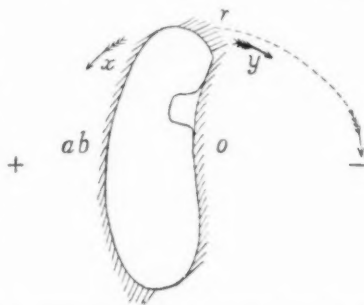
FIGURE 4. — Diagram showing Type II of reaction. References as in the previous figure.

wards the cathode. This method of reaction evidently forms a distinct type which I have called Type III. In the second method of reaction, that shown by the last 15 per cent of the animals occupying the defined positions, the Colpidium starts off slowly in the curved path towards the cathode, but after swimming for a short distance, jerks towards the aboral side, without rotating on the long axis, thus increasing the arc separating its anterior end from the cathode. It then starts ahead in the curved path once more. These movements, namely, swimming ahead in a curved path, more or less transverse to the direction of the current, followed by a jerk towards the aboral side, are repeated till finally the animal finds itself

FIGURE 5. — Diagram showing the path which Colpidium takes in swimming to the cathode.

with the long axis in the direction of the current, but with the anterior end towards the anode. The next jerk to the aboral side carries the animal around so that the aboral side is nearest the cathode, and then it simply swings around as is usual for an individual in such a position, according to the reaction of Type I, till the anterior end is

towards the cathode. This method of reaction constitutes Type IV (Fig. 6).

If the angle formed by the long axis of the body with the line of direction of the current is greater than 90°, and the *oral* side is towards the cathode (Fig. 1, positions *B* to *D*), the exact form of reaction depends as before on the amount of that angle. When the angle is between 90° and about 130° (Fig. 1, positions *B* to *C*), the reaction in the majority of cases consists in swimming slowly in a line somewhat transverse to the current and curved towards the cathode and the oral side, till finally the animal comes around into orientation. This is evidently the reaction of Type II (Fig. 4). Besides this reaction of Type II, Colpidia in these positions show reactions of Type III and Type IV. The difference shown by animals in the positions between *B* and *C*, from those in positions *A* to *B*, is in the relative frequency of occurrence of the types. In the former group, Type IV is relatively much more, and Type III relatively much less frequent than in the latter. Type II also occurs less frequently among individuals of the former group.

If the angle formed is between 130° and 180° (Fig. 1, positions *C* to *D*), the reaction is usually that of Type I, although Type IV (Fig. 6) frequently occurs.

FIGURE 6. — Diagram showing Type IV of reaction: *o*, oral side; *ab*, aboral side; 1, 2, 3, 4, and 5, different positions taken by the animal in this type of reaction.

Probably about 80 per cent of the reactions of animals in these positions are of Type I (Fig. 3).

In all these cases if the animal is swimming at the time of turning on the current in such a direction as to make with the line of current any of the angles just discussed, it simply stops at once and reacts as has been described for a quiet animal.

In all these reactions there are, under given conditions, individual variations in the type taken, but some one of those described is always followed.

Weak and Strong Currents. — In case a weak current is used, the only difference observed is that fewer animals are affected by it, and in fact in such a current the majority of the animals do not swim to the cathode, but keep on their usual paths. With a weak current, there is a tendency for animals in all positions to react according to Type I (Fig. 3), that is, by turning to the aboral side. In the path taken by the animal in swimming to the cathode under the action of a weak current, the angles formed are very obtuse, that is, the path is more nearly a straight line.

If the current acting be a strong one, in the great majority of cases the reaction of an animal in any position from *A* to *C* (Fig. 1) is that termed Type II, and in positions from *C* to *D* (Fig. 1), Type IV becomes, with relation to Type I, more frequent than in a current of medium strength. With strong currents, the angles in the path are more and more acute as the intensity of current increases. The animals often apparently swim backwards, as has been described by Ludloff [1] for Paramecium. The significance of this will be discussed later. It is very difficult to get a current of sufficient strength to cause Colpidium to disintegrate at the anode, as it is usually killed by a strong current without any such breaking down or granular disintegration of the protoplasm.

With all current strengths, on opening the circuit the animal immediately turns to the aboral side and swims away; that is, it goes through a typical motor reflex. This shows that there is a stimulation at breaking.

Changes in Body Form. — There are certain changes of form which, while they do not possess the striking character of those seen in Paramecium and some of the Hypotricha, are still evident. It may be observed that a Colpidium, lying with its long axis in the direction of the current, tends to swell at the cathode end, and to become smaller, or apparently contracted, at the anode end. This same fact may be observed if the animal is in a position at right angles to the direction of the current. In this case the cathode half of the body is seen to lengthen slightly, at the same time becoming more convex, while the anode side shortens, tending to become somewhat concave. This produces a slight bend in the animal with the convexity towards

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[1] LUDLOFF: Archiv f. d. ges. Physiol., 1895, lix, p. 525.

the cathode. Such effects may sometimes be seen in animals ex-
amined in water, but are much better seen when thin gelatine is used
so that the specimen remains alive but cannot move. This apparent
contraction and expansion will be discussed more fully later. The
same phenomenon has been described in a recent paper by Carlgren [1]
as taking place in dead specimens of Colpidium and several other
animals.

Relation of the Cilia to the Stimulus. — The movements of the cilia
in the different positions of the animal were studied both by direct
observation of the cilia themselves, with the animal in a thin gelatine
solution, and by means of the currents set up in the surrounding
medium. These currents could easily be followed from the motion
of indigo granules suspended in the water. These two methods gave
the same results.

The relation of the ciliary movement under the action of the
current may be summed up in a general statement, similar to that
given by Ludloff (*loc. cit.*) for Paramecium. In general it may be
stated that, *whatever the position of the animal, if a plane perpendicular
to the line of direction of the current be passed through the geometrical
central point of the infusorian, all the cilia on the cathode side of that
plane will beat stronger, that is, have their effective stroke, towards the
anterior end of the body ; and all the cilia on the anode side of such
a plane will have their effective stroke towards the posterior end of the
body.* These positions and movements of the general body cilia, I
have called the " forced positions " and " forced movements " for
reasons which will be explained later. These movements will perhaps
be made clearer by an illustration. If, for example, the cilia on the
cathode side of a Colpidium lying in thin gelatine at right angles
to the current be observed, they will be seen to point towards the
anterior end of the body, and to be beating in comparatively short
strokes. The angle included between the cilium in its most posterior
position and the surface of the body is about 45° or even less. On
reversing the current, the whole row of cilia move backwards together
till they come again to form an angle of 45° with the surface of the
body, but now pointing towards the posterior end. The absolute
regularity of this movement is remarkable ; the distance between
symmetrical points on any two or more cilia does not change in
the slightest degree. The appearance is as if all the cilia in a row

[1] CARLGREN: Ueber die Einwirkung des constanten galvanischen Stromes auf
niedere Organismen. Archiv für Physiologie, 1900, pp. 49–76.

were connected by a rod, and when the current is reversed it is as if this rod were pulled backwards by some invisible force. Exactly the same phenomena take place on the anode side, except that all the cilia point in the opposite direction, that is, towards the posterior end of the body (Figs. 1, 2, 3, 4, 5, and 6).

In addition to this effect on the general cilia of the body, that upon a special group of cilia must be considered. These are the cilia covering the very anterior end of the body (Fig. 2, *r*). They beat backward when the animal is unstimulated, but on stimulation by the current they beat strongly towards the oral side, in every position of the animal except when the anterior end is towards the cathode, thus tending to cause the animal to turn aborally, as it usually does on the application of a chemical or mechanical stimulus (*vide* Jennings, *loc. cit.*). These cilia are probably the ones which are instrumental in bringing about the usual motor reflex of the animal in response to chemicals, etc. The action of these cilia can best be seen in a preparation in which indigo is suspended in the water. Upon making the current, it will be seen that a space on the oral side of the body, just in front of the mouth, becomes clear of granules, while a stream of granules rushes around the anterior end, and another passes up or down the oral side to a point a little in front of the mouth, swerving there on account of the eddy caused by the rapid current around the extreme anterior end.

Movement of Granules in the Body.— If the Colpidia are examined in a thin gelatine solution under a high power objective, a definite movement of the granules in the body can be observed when the current is made. This movement of the granules in the protoplasm is in the direction opposite to that in which the cilia on the same side of the body point as a consequence of their forced position. For example, the cilia on the cathode side of the animal point towards the anterior end, while the granules in the protoplasm on the same side move, on closing the circuit, towards the posterior end of the body. On the anode side the granules move towards the anterior end. On reversing the current the granules immediately start in the opposite direction. This can be observed most satisfactorily when the animal is at right angles to the direction of the current. Here, on closing, the granules in the two halves of the body start to move in opposite directions, leaving a line down the middle of the body in which there is no movement. The movement of granules is slight in Colpidium, and consists in a shifting in position for a short distance only, in one

direction or the other.   It is much more distinct and pronounced in the Hypotricha.   The relation of this movement to the cilia is noteworthy.   The two movements under the action of the current are exactly simultaneous and synchronous, that is, on reversing the current the granules move just as fast in one direction as the cilia do in the other.   The cilia seem like stiff rods with their inner ends projecting into a granular mass of protoplasm, and when the current is made the mass of protoplasm moves in one direction or the other apparently pulling with it the bases of the cilia, so that their free ends point in the opposite direction.

Results from Colpidium. — There are thus in Colpidium four types of reaction to the stimulus of the electric current to be distinguished. Type I (Fig. 3) is evidently, in large part at any rate, the usual motor reflex of the animal, and carries it into a position where either it is less stimulated or else is for some reason unable to leave this position or orientation so long as the current acts.   It can be readily seen however that in addition to the cilia of the anterior end, or, as they will for convenience be called, the *reflex cilia* (Fig. 3 *r*), turning the animal according to this type, the forced movements of the body cilia must help in this rotation.   In other words, the two forces, motor reflex and forced movement of body cilia, act together to produce the same result in the reaction of Type I.   For this reason orientation to the current is more quickly brought about by this type of reaction than any other.

The second type of reaction (Fig. 4) is a very slow one, and in it the animal appears as if it were being dragged around by some force outside itself, or else attempting to turn against great resistance. The reason for this slowness and apparent difficulty of movement is seen in the fact that here the reflex cilia and the general body cilia in their forced movement are working in opposite directions.   The reflex cilia are beating in such a way as would ordinarily turn the animal towards the aboral side, while the forced movements of the general body cilia are of a sort which, if unopposed, would turn the body towards the cathode, — in this case towards the oral side. These two forces act simultaneously, and it is evident that the stronger will determine which way the animal as a whole shall turn. The force of the reflex cilia becomes weaker the nearer the animal comes into orientation with the anterior end to the cathode, so when the angle formed by the axis of the body with the line of direction of the current is small, and the oral side is towards the cathode, the

reaction is always of Type II. That the action of the reflex cilia becomes weaker is seen from the fact that under direct observation these cilia are straightened out and beat weakly and spasmodically when the animal is directly oriented towards the cathode. This condition is arrived at gradually in the turning, although the observation of the weakening in stroke is not so easy here as in other larger forms, such as the Hypotricha and Stentor.

Type III is a form of reaction peculiar to Colpidium, and really begins according to Type II, after which the animal turns to the aboral side, as in Type I. This does not uniformly take place, and seems to occur only when an animal in reacting according to Type II chances for some reason to rotate on the long axis. Then of course reflex cilia and body cilia act together as in Type I, and the animal quickly turns towards the cathode.

Type IV (Fig. 6) shows again the struggle between motor reflex and forced movement. During the first part of the reaction the forces are nearly evenly balanced, but the reflex force is slightly more powerful than the other, so that eventually the animal is turned towards the aboral side far enough for the two factors to act together, and then becomes quickly oriented.

The significance of the existence of these types of reaction to the electric current is evidently that in the action of the current there is, besides the stimulation of the organism as a whole tending to produce a motor reflex, a forced movement of the body brought about by a change in the position and in the field of action of the cilia. This change in position and consequent change in effective stroke of the body cilia is evidently something entirely distinct from the usual motor reflex of the animal, because both can be observed to take place at the same time. I have called the change in position of the cilia a "forced movement," because it resembles an ordinary physiological "forced movement" in that it continues to act in the same way as long as the stimulating agent remains the same. Forced movements and positions of the cilia exactly like these are caused in infusoria, so far as I know, only by the electric current.

OXYTRICHA FALLAX STEIN.

When a current of medium strength is sent through a layer of water containing Oxytricha, they nearly all orient themselves immediately with the anterior end towards the cathode, and either swim slowly in this direction, or remain quiet at the point where they

became oriented. The number of Oxytricha individuals which immediately become oriented is relatively larger than in the case of Colpidium.

The swimming to the cathode is slow, and many of the individuals after becoming oriented simply remain quiet.

Types of Reaction. — Oxytricha conforms in its reactions to some of the same general types which were described for Colpidium, but not to all of them. The reactions for the different positions of the animal are briefly as follows:

When the Oxytricha is lying or swimming at the moment of making, with the anterior end towards the cathode, it either stops and becomes fixed in position, or swims slowly towards the cathode.

If the animal at making has the anterior end directed towards the anode, it orients itself immediately, and invariably by a reaction of Type I (Fig. 3), that is, it whirls on the short axis of the body towards the *right* till its anterior end is towards the cathode.[1] This is the same type of reaction by which it responds to other stimuli, and here, as in Colpidium, the reflex cilia and the general body cilia, in their forced movement, work together.

When the animal is in any of the positions from *A* to *B* (Fig. 1) it reacts in one of two ways, the method taken being dependent on some undiscovered individual difference. The majority of the individuals react from this position according to Type IV (Fig. 6) with the reflex component in the movement relatively strong. That is, the animal moves slowly in a path diagonal to the direction of the current, at intervals turning to the right side, till eventually it turns completely around in that direction and gets the anterior end towards the cathode. The rest of the individuals react according to Type I, — that is, by turning towards the right side, in some cases the anterior end of the body describing an arc as large as 345°.

Individuals in positions *B* to *C* (Fig. 1) react in about equal

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[1] In the references to the figures in the descriptions following that of Colpidium, it is to be understood that, in the figure referred to, the outline of the animal under consideration is supposed to be placed in the same relative position as is that of Colpidium in the actual figure; that is, with the anterior end directed in the same way, and the structurally defined side towards which the animal turns in its usual motor reflex in the same relative position as the aboral side in the figures of Colpidium. Thus the sides corresponding to the aboral in Colpidium are for the different infusoria as follows: Oxytricha and the Hypotricha, the *right;* Paramecium, the *aboral;* Stentor, the *right;* Spirostomum, the *aboral;* Chilomonas, the *side bearing the lower lip.*

numbers according to Type I or Type IV. Those in all other positions react according to Type I.

Effect of Breaking and Reversing the Current. — In all cases, and with all current strengths, when the current is broken Oxytricha gives a sharp motor reflex without, in most cases, swimming backwards, that is, it turns to the right and swims away.

The animal always reacts in the same way when the current is *reversed*, by turning on the short axis of the body towards the right side till the anterior end is directed to the new cathode.

Movements of the Cilia. — The general body cilia are thrown into exactly the same forced movements by the current in Oxytricha as in Colpidium, that is, those on the cathode surface of the body beat towards the anterior end, and those on the anode surface towards the posterior end.

The " reflex cilia" in this case are, as before, the cilia at the anterior end of the body. These too are affected in the same way by the current as the corresponding cilia in Colpidium. Thus in all positions, except when the anterior end is towards the cathode, these cilia are beating violently in such a way as to turn the animal towards the right side. When the anterior end is towards the cathode, these cilia are stretched out straight in front and nearly motionless.

On reversing the current the body and reflex cilia change their positions with the same promptness and regularity as in Colpidium.

Movements of the Granules in the Body. — The movements of the granules in the protoplasm under the influence of the current can be very clearly and easily observed in the case of Oxytricha. Here, as before, the granules on each side of the body move in the opposite direction to that taken by the cilia in their forced movement. That is, the granules on the cathode side of the body move towards the posterior end and those on the anode side towards the anterior end. The connection of the movement of the granules with that of the cilia is very apparent here. The rate of movement in the two is exactly the same on reversing, and the appearance is exactly as if the protoplasm were changing its relative position in order to move the cilia.

This movement of the granules in the living body is evidently different from the carrying of the granules to the anode surface by the cataphoric action described by Carlgren[1] as occurring in dead

[1] CARLGREN: Archiv für Physiologie, 1900, pp. 49-76.

animals when a strong current is used. The phenomenon described here evidently requires that the protoplasm be alive, and whatever the force causing the movement, it must be of sufficient power to overcome the tendency of the granules to go to the anode surface of the body as a result of the cataphoric action. The phenomenon depends also on the structure of the body, for whatever be the animal's position with reference to the current, the granules cannot during life be induced to go in any direction except parallel to the long axis of the body and either towards or away from the anterior end.

Changes in Form of Body. — The changes in the body form are very distinct in Oxytricha. In general terms the changes are those described by Carlgren for other forms, and consist of an apparent contraction on the anode and expansion on the cathode side proved by him to be due to the cataphoric action carrying the fluid in the body to the cathode surface. There are, however, certain differences depending on the fact that the animal is alive. The posterior end and right side of the body are apparently much less dense and more mobile than the anterior end and left side. The effect of this difference is evident under the action of the current. When the animal lies at right angles to the direction of the current, with the right side of the body towards the cathode, the appearance is as if the whole body lengthened out slightly. When on the other hand the right side of the body is towards the anode, the whole body seems to contract, though the contraction appears one-sided, as did the expansion in the other case. When the animal lies with the anterior end towards the anode, the body, as a whole, lengthens, though all the apparent expansion is at the posterior end, and, vice versa, when the anterior end is towards the cathode, the body as a whole shortens, but, in this case, as before, the contraction is at the posterior end. Thus it is seen that in all positions the anterior end and left side of the body do not change their contour at all or only very slightly, while the posterior end and right side change a great deal. From these facts, in connection with the work on other infusoria, it seems certain that the seeming difference from Carlgren's results is due simply to structural relations of the animals. With a strong current, the anterior end and left side begin to show slight expansions and contractions. It is very certain here that when the current is flowing the cataphoric action causes a movement of the fluid in the body to the cathode surface, and this side becomes swelled out, or tends to

become so, being prevented in certain cases by structural relations, and at the same time the anode surface becomes crumpled or contracted, or tends to become so.

I have emphasized this point of the slight variation in this phenomenon from that described by Carlgren as typical, because at first sight the two appear to be very different, and it seems of importance to keep in mind the fact that the typical scheme for the change of body form may be in any case more or less modified by structural peculiarities of the animal considered, without in the least detracting from the validity of the scheme.

Weak and Strong Currents. — In a very weak current Oxytricha is not affected at all. The first current to affect the animal sets into activity the reflex cilia, without yet being strong enough to cause the forced movements of the body cilia. The reflex mechanism here is evidently more sensitive than the general body of the animal, as evidenced by ciliary movement. The result of making a current of this minimum effective strength is that the animal turns sharply to the right till its anterior end is towards the cathode and there it stops. Here we see the orientation of an animal by means of a motor reflex to a constant stimulus acting in a straight line. From this strength at which the current begins to be effective up to a strength sufficient to cause the animal to go to pieces, the essential results are the same in all cases. In a current slightly stronger than the minimum effective one, the forced movements of the body cilia begin and continue throughout. With a strong current the protoplasm of the animal undergoes granular disintegration.

OTHER HYPOTRICHA.

Besides Oxytricha, several other Hypotricha were studied without determining the species, and the results obtained from all were essentially the same. In some of the forms, particular effects could be more clearly made out than in Oxytricha, but the differences were of degree only.

In one form the reaction when the animal was in any position from *A* to *C* (Fig. 1) was very interesting. Here the motor reflex factor and the forced movement were so equally balanced that the animal, instead of becoming oriented as usual by a reaction of either Type I or Type IV, did not become oriented with the anterior end towards the cathode, but, starting to react according to Type IV, it continued to swim in a path diagonal to the current and was never able to turn

completely around. From all other positions it reacted according to Type I as usual. This case shows the possible effect of the two factors in these reactions.

From the number of forms studied, I think it may safely be said that all the Hypotricha show essentially the same type of reaction to the current, although there may be slight individual differences due to various causes.

RESULTS FROM OXYTRICHA AND THE HYPOTRICHA IN GENERAL.

The Hypotricha are seen to respond to the current by one of two types of reaction which were described for Colpidium. In both of these reactions, two factors can be distinguished, one due to " forced movements " of the general body cilia, and the other due to an excitation of the motor reflex mechanism. This latter factor is relatively more important in determining the type of reaction in the Hypotricha than in Colpidium.

PARAMECIUM CAUDATUM EHR.

In my study of Paramecium I was able to confirm the work of Ludloff[1] in every particular.

Type of Reaction. — At the time Ludloff's work was done, the motor reflex plan of reaction in the Protozoa was unknown, and consequently he makes no direct mention in his paper as to whether the animal does or does not turn towards a structurally defined side. It would seem, however, from his description, very probable that it did not turn towards a structurally defined side in becoming oriented. This I find to be the case. Paramecium turns, in orienting itself to the current, according to the direction in which it happens to lie at the moment of making, without regard to a structurally defined side. It turns simply in the direction towards which the forced movements of the general body cilia carry it, that is, through the shortest path that will bring the anterior end towards the cathode. The reaction is of the Type IV with the motor reflex factor reduced to its lowest terms. That this factor is, however, still present seems almost certain from the following facts. First, that the spirals of the path taken by the animal in swimming to the cathode become more closely packed together with increasing current strength, and this was seen in the case of Colpidium to be due to the motor reflex component of the reaction. Secondly, when the Paramecia are placed in gelatine

[1] LUDLOFF: Archiv f. d. ges. Physiol., 1895, lix, p. 525.

solution, so that they can move but very slowly, it is found that in every position, except with the anterior end directly towards the cathode and the long axis of the body in line with the current, the animals rotate about their long axes. When in exact orientation, with anterior end towards the cathode, there is no rotation, though the forced movements of the body cilia continue as before. This rotation about the long axis is probably the necessary mechanical result of the beating of the reflex cilia when the animal is so held that it cannot turn about its short axis. The reasons why these reflex cilia are unable to influence the reaction more, are probably the shape of the body, the relatively small number of the reflex cilia as compared with the general body cilia, and the much greater relative strength of the latter.

Effect of Opening and Reversing. — On opening the current the Paramecia all turn more or less, as the case may be, to the aboral side (thus giving the typical motor reflex) and swim away. On reversing the current, the animals become oriented towards the new cathode as when the current was first made. They continue turning in whatever way they happen to be started at the time of reversing.

Movements of Cilia. — In the effect of the current on the body cilia, I found exactly the same movements as in the case of Colpidium and the Hypotricha, and as described by Ludloff for Paramecium. The effect on the cilia causing the motor reflex has been discussed.

Movements of Granules. — The movements shown by the granules in the body are the same as those described for Colpidium and the Hypotricha. The granules move in the direction opposite to the cilia in their forced positions.

Changes in the Body Form. — The changes which take place in the form of the body under the action of very strong currents have been carefully described by several observers. These changes consist of a swelling at the cathode and a contracting to a point at the anode end of the body, eventually leading to a breaking down of the protoplasm and the death of the animal. Under the action of a current of medium strength, I have found that the same changes take place as in Colpidium and the Hypotricha, as noted by Carl gren. The cathode side or end swells out slightly, and the anode appears to contract. I find in Paramecium differences due to the structure of the body comparable to those found in Oxytricha, that is, the oral side and anterior end change less and evidently have greater firmness than the aboral side and posterior end.

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Effect of Strong and Weak Currents. — With an increasing current strength, the most important changes in the reaction of Paramecium are a decrease in the speed of swimming to the cathode, and with the very strong currents, an apparent swimming backwards towards the anode. The effect of weak currents is the same as that of currents of medium strength, except that fewer animals are affected.

STENTOR COERULEUS EHR.

Sense of Reaction. — In Stentor the sense of reaction is cathodic, and a relatively larger number of animals are immediately affected by currents of all strengths than in any of the infusoria previously described. In fact, practically all the Stentors immediately orient themselves and swim towards the cathode after the current is made. The movement to the cathode is slower than the normal swimming.

Type of Reaction. — The method of orientation taken by Stentor is rotation on the transverse axis in the way which will turn the anterior end towards the cathode by the shortest path. If at the moment of closing, it happens to lie so that the body forms some angle with the line of current, it turns in the way the forced movements of the body cilia would carry it. If at closing, it lies with the anterior end towards the anode and perfectly in line with the current, it swims ahead for a short distance till it gets out of that alignment, then turns as before. The movement towards the cathode is generally in a slightly diagonal direction, probably due to the asymmetry of the body. The reaction here is evidently of the Type IV, with the motor reflex factor entirely absent, or at any rate of such slight importance as to have no effect on the movement.

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FIG. 7. — Diagram showing the relation of the adoral cilia in Stentor when the animal is at right angles to the current.

Effect of Opening and Reversing the Current. — On opening the current the only effect observable is that the animal lengthens out from the contracted position which it assumed while the current acted, and swims away. There is no evidence of the animal being stimulated to a motor reaction by opening the current as in the other cases.

On reversing the current, the infusorian immediately begins to orient itself to the new cathode in exactly the same way as when the current was first made.

Movement of Cilia. — The forced movements of the general body

cilia are here exactly those previously described. The adoral cilia, on account of their greater size and stronger beat, are the principal factors in turning the animal into orientation. That their movement is not of the nature of the usual motor reaction type is certain from the fact that they beat differently as they form different angles with the direction of the current. That is, the cilia on that part of the circle nearest the anode beat backwards, and those nearest the cathode beat forwards, towards the anterior end of the body. When the animal is oriented, these cilia point straight to the front, and beat only spasmodically.

These movements of the adoral cilia are, when analyzed, evidently not essentially unlike the forced movements of the general body cilia, the only difference being that the adoral cilia are large and their individual movements can be distinctly followed.

Movements of Granules. — The movements of the granules in the body were found to agree with those described in the other cases. The movements do not seem to include all the granules, but rather to be confined to a layer next the surface of the body. There may be, however, and probably are, movements of the granules in the whole body, but these are less marked near the centre.

Changes in Body Form. — When a current is made, the body of the Stentor always contracts more or less, as long as the current acts. This is probably due to a stimulation of the myonemes.

SPIROSTOMUM AMBIGUUM EHR.

The sense of the reaction here is "transverse," as first described by Verworn.[1] That is, after a current has been acting for a time on a preparation of Spirostoma, all of them are found to be lying, or slowly swimming, with the long axis of the body at right angles to the direction of the current. When in this position, there is usually a great deal of twisting and bending of the body, and the swimming, if the animal is swimming at all, is very slow.

Method of Orientation. — The method of orientation here is very indefinite and indeterminate. I have never seen any two Spirostoma get into a position transverse to the current in exactly the same way. There is always more or less bending and twisting of the body, and finally the animal reaches an approximately transverse position, and then straightens out, although in most instances it

[1] VERWORN: Archiv f. d. ges. Physiol., 1896, lxii, pp. 415–450.

soon begins again to double and fold on itself, keeping in a general transverse direction. There is no evidence of the animal's usual motor reflex anywhere in the reaction. It gets into a certain position and stays there, probably because it is unable to move out of it while the current acts. Evidently the reaction of Spirostomum does not agree with any of the types described for other forms, but is peculiar to the animal itself.

Effect of Opening and Reversing Current. — There is no evidence of a motor reflex being produced by breaking the circuit.

If the current is reversed after the Spirostomum is oriented, it either may contract sharply and then begin to twist and bend on itself till the anterior end is pointed in an opposite direction, or it may come back to the same position that it occupied before. Sometimes the reversing causes only a sudden contraction, and in a few cases not even that. Changes in the ciliary movement invariably accompany reversal.

Movements of Cilia. — Forced movements of the body cilia essentially the same as those described for other forms are set up by the current. The cathode cilia beat towards the anterior end, and the anode towards the posterior. These movements are strong and easily seen till the animal gets into the transverse orientation, when they become very slow and finally stop altogether on both sides of the body, or only on the cathode side, while a weak spasmodic movement continues on the anode side. When the current is reversed, the cilia change their direction, and if the animal is not yet in its transverse position, they beat in the opposite direction, but if it is oriented and the ciliary movement has stopped, reversing causes the cilia to point in the opposite direction.

Up to a certain point the ciliary movements of Spirostomum are exactly the same as in other forms; but when it comes into the transverse position, the animal apparently becomes paralyzed, as far as movement of the cilia goes, by the action of the current. This is much the same as with the adoral cilia of Stentor and the reflex cilia of other forms, when they are directed towards the cathode.

It will be seen that while the "forced movements" of the cilia caused by the current are the same in Spirostomum as in other forms, the resulting orientation is different. It is therefore evident that this forced movement is not the only factor in causing orientation in this case.

Movements of Granules and Changes in Body Form. — In respect

to these matters, Spirostomum shows essentially the same phenomena as Oxytricha and the other infusoria described.

Different Current Strengths. — With strong currents, Spirostomum immediately begins to go to pieces, contracting rhythmically in whatever position it may be with reference to the current, without attempting orientation. A current of medium strength has the same effect in causing the body to disintegrate if it acts for some time. A weak current, if strong enough to be effective at all, acts like one of medium strength.

CHILOMONAS PARAMECIUM EHR.

The sense of reaction in Chilomonas has been described by Verworn [1] as anodic. This I have not been able to confirm, as I find that under conditions which are evidently best fitted to show the sense of the true electrotactic response, this flagellate swims actively to the cathode. The first appearance of a preparation under the influence of the current is as if the animals were swimming to the anode, but careful study reveals the fact that with the proper current strength the animals swim to the cathode actively, and when they seem to swim towards the anode it is because they are being carried by the cataphoric action of the current. The reaction of the animals to different current strengths in detail is as follows.

With a very weak current of a strength just sufficient to affect the animals, which strength varies with Chilomonads from different cultures, all the animals that are not attached by a flagellum to the slide or cover glass, orient themselves with the anterior end towards the cathode and swim towards it.

With a somewhat stronger current, all the animals are seen to swim violently towards the anode, with their long axes still in line with the current. Close examination shows, however, that the anterior end is *still directed towards the cathode*. The appearance is as if they were swimming backwards towards the anode. That they are really endeavoring to swim towards the cathode, and are being carried backward by a superior force, is evidenced by the fact that the movement is slower than the usual swimming movement, and also slower than that of the granules, bacteria, etc., in the fluid, which are being carried by the cataphoric action unhampered. Besides, if the strength of the current be grad-

[1] VERWORN: Archiv f. d. ges. Physiol., 1889, xlvi, pp. 267–302.

ually reduced, a point is reached at which the force of the swim-ming movement and the cataphoric action balance each other, and the animal moves only slightly either way, the flagellum keeping up an energetic beating all the while. If the current be still more decreased, the animal, while the flagellum still beats forcibly, swims again towards the cathode. There is no change in the motion, but it again becomes stronger than the cataphoric action, thus per-mitting the animal to go towards the cathode.

With stronger currents, the animals are carried more and more rapidly till finally they are unable to remain oriented towards the cathode, and are carried with anterior end towards the anode, sidewise, end-over-end, and in all ways.

For orientation with the anterior end towards the cathode, the ani-mals turned towards the lower lip, in all cases which I was able to observe clearly. Therefore it seems extremely probable that the animal always becomes oriented to the current by turning towards the lower lip in the regular reflex way. This is what might be expected, for there are no cilia here in which a regular forced move-ment could be set up, and by analogy from the ciliates, if any forced movement of the flagellum were started, it would agree in form with the usual motor reflex.

IV. ANALYSIS OF OBSERVATIONS.

As was stated at the beginning of the paper, the problem in view in this study was embodied in this question: "Do the Protozoa react in the same way to electrical as to other stimuli, and how is the reaction to the current brought about?" In regard to the first part of this question I think the answer may now be definitely given. The infusoria examined do not react in exactly the same way to the current as they do to other stimuli, so far as is known at present. The thermotactic, tonotactic, thigmotactic, and chemotactic reac-tions, as well as the reaction to mechanical stimuli, have entirely, or in part, been worked out for each of the infusoria whose reaction to the current I have studied. The response to the electric current is different from that to any of the other stimuli. In general terms the reaction of these organisms to other stimuli, chemical, for exam-ple, takes the form of the "motor reflex" described by Jennings, in which the animal turns towards a structurally defined side after

[1] JENNINGS: This journal, 1900, iii, p. 220.

stimulation. The reaction to the current does not have this definiteness, but the exact form of response varies with the position which the animal at the moment happens to occupy. Thus in Colpidium, for example, while the current remains constant, the reaction is either (*a*) of the usual sort, that is, a turning towards the aboral side, as, for instance, in response to a mechanical stimulus (Type I); or (*b*) of a sort which represents a struggle between the usual reflex and what has been called the forced movement of the general body cilia (Type IV); or, (*c*) one the form of which is determined entirely by this forced movement factor. Now these forms of reaction depending on different positions of the animal have been found in the other animals studied with, of course, variations in the different cases. In the several species the relative value of these two factors in determining the form of reaction varies greatly from Stentor, in which the reflex factor is at its very lowest terms, or perhaps entirely absent, up through Paramecium, the Hypotricha, and Colpidium, in each of which its relative importance increases, to Chilomonas where, so far as has been determined, there is no forced movement factor in the same sense that the term has been used for the ciliates. In all the cases except that of Chilomonas, the reaction to the current differs essentially from the usual motor reflex.

The reaction of the animals studied (excepting Spirostomum) may be reduced to a general statement holding good for Chilomonas and Stentor, if these were considered the opposite ends of a series, with one factor very much reduced in each case. Such a statement would be: The reaction to the constant current is brought about by a graded combination of two factors, one of a forced nature possibly not necessarily dependent on a stimulation of the animal as a whole, and the other of a reflex nature. The forced movement factor is the result of a definite movement of the cilia of the body, depending for its direction on the direction of the current, as has been described in detail. The second factor, which I have called the reflex factor, is the result of the movement of a certain set of cilia which tend to turn the animal always in one direction. This factor, so far as the evidence now shows, is an expression of the attempt of the animal to react in the ordinary reflex way.

This statement I wish to make general merely for the species examined. It seems fairly probable that, in view of the fact that the forms studied represent a wide range of structure, some such statement will hold at any rate for all ciliate infusoria.

The forced movement factor in the reaction is that which Ludloff described for Paramecium. I have been able to confirm completely his results and to extend them to several other ciliates. He found that under the action of the current all the cilia on the cathode surface of the body point towards the anterior end, and all those on the anode surface towards the posterior end. This relation I have found in all the ciliates examined, considering the general body cilia. These positions, in general, persist as long as the current acts, although the beats may spasmodically change for an instant. If, however, the current be sufficiently increased, these spasmodic changes will not take place, and their occurrence is rare in any event. Ludloff's observations were partially confirmed for Paramecium by Jennings,[1] who found that the current acting on thigmotactic individuals caused the cathode reversal of cilia, although the cilia soon after went back to their normal beat, probably on account of the relatively greater strength of the thigmotactic response.

As Ludloff worked only on Paramecium in which the motor reflex factor in the electrotactic response does not determine the *form* of reaction, he very properly concluded that the forced movements of the general body cilia were the essential factor in orientation. I think I have shown that in other ciliates studied there is present in the reaction another factor which may equally determine the form of response. This reflex factor is present also in the reaction of Paramecium, only it is not of sufficient strength to be determinative. It is due to the current setting in action those cilia which cause the ordinary motor reflex, turning the animal towards a structurally defined side. This reflex factor may conceivably be either an attempt to react in the ordinary way to the stimulus caused by the electric current, with the prolonging of one part of the reaction (namely, that in which the animal turns to one side), or it may be a *forced movement* of structurally *definite* cilia exactly the same as the forced movement of the body cilia, the current differentially affecting a certain region of the body. The evidence now at hand seems to warrant the conclusion that it is in large part, at any rate, simply an attempt of the animal to react in the ordinary reflex way. Additional evidence may prove it to be a forced movement as I have suggested.

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[1] JENNINGS: Journal of physiology, 1897, xxi, pp. 258–322.

V. Theoretical Considerations.

The first well grounded attempt to explain *how* the current produced its effect on the organism was the "chemical" theory of Loeb and Budgett.[1] These authors hold that the current produces its effect only indirectly through the action of ions in the surrounding electrolyte. This theory has been criticised by Carlgren[2] from the point of view of the change in the form of the body produced by the current. Before seeing Carlgren's paper, other considerations led me to practically the same view in regard to the electrolytic theory. If the effect of the current is due merely to the action of the ions in the water, one would hardly expect to find any difference in kind between the form of reaction to these same ions in a weak solution and when separated by the action of the current. As a matter of fact, there is such a difference. To these ions in a solution the animals react in the motor reflex way; while, as I have shown, the reaction to the current is of a different form with an entirely new factor introduced. This fact alone seems to afford nearly conclusive evidence that the principal effect of the current on these organisms is not due to its electrolytic action. It may be that the current may have some internal electrolytic action of sufficient force to be effective, but there does not seem to be evidence enough at present to warrant a conclusion in regard to the matter.

That the effect of the current is due in large part to its cataphoric action is the conclusion reached by both Carlgren and Birukoff.[3] This cataphoric action has been described by Wiedemann,[4] Quincke,[5] du Bois-Reymond,[6] Munk,[7] and Braun[8] on a physical basis. Carlgren has shown that the swelling of the body on the cathode side and the

[1] Loeb and Budgett: Archiv f. d. ges. Physiol., 1897, lxv, pp. 518-535.

[2] Carlgren: Archiv für Physiologie, 1900, pp. 49-79.

[3] Birukoff: Untersuchungen über Galvanotaxis. Archiv f. d. ges. Physiol., 1900, lxxvii, pp. 555-585.

[4] Wiedemann: Poggendorff's Annalen der Physik und Chemie, 1852, lxxxvii, pp. 321-352.

[5] Quincke: Poggendorff's Annalen der Physik und Chemie, 1861, cxiii, pp. 513-598.

[6] Du Bois-Reymond: Monatsberichte der königlichen preussischen Akademie der Wissenschaften zu Berlin, 1860-61.

[7] Munk: Untersuchungen über das Wesen der Nervenerregung, Leipzig, 1868.

[8] Braun: Annalen der Physik und Chemie, 1897, n. F., lxiii, p. 324.

crumpling or apparent contracting on the anode side are due to the cataphoric action, since the same phenomena occur in dead as well as in living Volvox colonies and protozoan bodies. He does not attempt to make the cataphoric action the cause of all electrotactic phenomena, but says that the truth is probably "that the effect of the electric current on lower organisms is first of all a displacement of liquid in the interior of the body; liquid is carried away from the anodal side of the organism, thereby calling forth a stimulus to contraction, while conversely the streaming of the liquid to the cathodal side produces there a stimulus to expansion." With this view I agree. Now there are certain observations of Carlgren's which apparently differ from mine. He finds that the granules in the body in dead infusoria, and parthenogonidia in dead Volvox colonies move to the anode side, as would be expected from regular cataphoresis. On the other hand, I have described the granules in the bodies of living animals as moving always in the opposite direction to that towards which the cilia point in their forced positions. That is, in living animals the granules move under the action of the current in directions determined by the structural relations of the body. Moreover, the same phenomena of apparent expansion and contraction, due to cataphoric action, take place in living as well as dead animals, so that it seems fair to conclude that this movement of the granules in living animals is the expression of the *active* expansions and contractions of the body caused by the stimulus of the current. The forced positions of the cilia and the movement of the granules are certainly very closely related, since, as I have shown, they always occur simultaneously, and are in opposite directions. They are evidently not directly due to the effect of the cataphoric action, but, since they take place only in living animals, are connected with the irritability of the protoplasm. The exact way in which the forced position of the cilia is brought about is not as yet certain, but it is certain that there are present in the body conditions which depend on the effect of the current on irritable, contractile protoplasm and the movements of the granules, and that the forced positions of the cilia are expressions of this effect.

Cataphoric action can be used to explain certain other of the general phenomena of electrotaxis. It has been shown that in the case of Chilomonas the current may be controlled so as to cause the animal either to remain in practically the same place, or to be carried backward to the anode, while all the time it is oriented

towards the cathode, and swims violently in that direction. The fact that under the action of strong currents Paramecium moves backward to the anode is to be explained in a similar way. While this movement is taking place, the animal is very evidently swimming most vigorously, as it was while the current was weaker, only the cataphoric action has become of greater power than the beat of the cilia. This fact seems to promise a method whereby the swimming force of these Protozoa may be measured.

In certain of the infusoria which I studied, there was a stimulus to a motor reaction of the usual type at both the making and breaking of the current. The reaction at breaking is very characteristic, since it is then uninfluenced by any other force, and the animals on opening the current all turn more or less towards a structurally defined side and swim away.

VI. SUMMARY.

It may be well in closing to state the main results in a condensed form. It is to be understood that these conclusions while stated in a general form are intended to apply only to the species studied. They may not apply to other Protozoa, though it seems probable that they will.

1. The infusoria studied react to the electric current in a way distinctly different from that in which they react to other known stimuli.

2. The reaction to the electric current is brought about through the graded action of two factors, — a " forced movement " factor and a " motor reflex " factor.

3. The " forced movement " factor is due to the action of the cilia of certain regions of the body which, so long as the current passes, occupy definite positions. In these positions the cilia on the cathode surface point towards the anterior end of the body, and those on the anode surface point towards the posterior end.

4. The " motor reflex " factor is due to the action of certain cilia which tend to cause the animal to turn towards a structurally defined side.

5. The effect of the electric current on these animals is not at all, or only to a very slight extent, due to its electrolytic decomposition of the surrounding electrolyte.

6. The cataphoric action plays a very considerable part in the effect of the electric current on living animals.

A PLETHYSMOGRAPHIC STUDY OF THE VASCULAR CONDITIONS DURING HYPNOTIC SLEEP.

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CONTENTS.

INTRODUCTION.

THE principal object of these experiments has been to determine the changes occurring in the volume of the arm as a consequence of hypnotic sleep and suggestion, and to compare the results so obtained with the observations which have been made by the same methods on normal sleep. It has been shown by Mosso,[1] Howell,[2] and other investigators, by means of the water plethysmograph, that the volume of the arm is increased during normal sleep. The same authors have also shown that mental and muscular activity cause a constriction of the arm. These changes are assumed by Howell to be due to vasomotor changes in the cutaneous blood vessels

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[1] Mosso: Ueber den Kreislauf des Blutes im menschlichen Gehirn, Berlin, 1881; Die Temperatur des Gehirns, Berlin, 1894.

[2] Howell: Journal of experimental medicine, 1897, ii, p. 313.

and a consequent alteration in the amount of blood flowing through the peripheral vessels. The present experiments have been extended so as to include not only the plethysmographic records, but records of the blood-pressure, pulse, respiration, and temperature, as well.

DESCRIPTION OF THE APPARATUS.

The plethysmograph used was similar to that previously described by Howell.[1] It consisted of a glass cylinder of sufficient size to allow the hand and a portion of the fore-arm to be inserted in it. One end of the cylinder was drawn out and was connected to one arm of a three-way stop-cock by stiff rubber tubing. On one side of the cylinder there was a small neck, into which was fitted a piece of glass tubing provided with a stop-cock. This opening served as an escape for the air while the apparatus was being filled with water. The two remaining arms of the three-way stop-cock were joined, one to the recording apparatus and one to the reservoir, which contained water for filling the apparatus. By turning the cock, the plethysmograph could be connected with the recording apparatus, the reservoir, or with both.

The recorder used was the form devised by Bowditch.[2] It consisted of a test-tube swung on a spiral spring in such a manner that the height of the water always remained constant, the test-tube being pulled up by the tension of the spring as water was withdrawn from the tube, and the spring, in turn, being stretched out as water was poured into the test-tube. The spiral spring was fastened to a short vertical rod, and this was attached to another longer vertical rod by means of a universal joint. The longer vertical rod was firmly fastened to a table. By means of the universal joint the height of the test-tube and the tension of the spring were very easily regulated. A pen of thin paper was attached to the test-tube and wrote against the blackened surface of a drum kymographion, which revolved once in six hours. Two other pens were arranged to write in the same vertical line. One of these pens was connected with an electric signal, in circuit with a clock, and marked intervals of one minute. The other pen was attached to a lever and was used to record the application of any stimulus that was given to the subject, or any change that was noticed in his condition.

[1] HOWELL: Journal of experimental medicine, 1897, ii, p. 313.
[2] BOWDITCH: Proceedings of the American Academy, May 14, 1866.

To keep the arm immovable in the plethysmograph, the device described by Howell[1] and Shields[2] was used. This consisted of a hinged collar of hard rubber, which fitted around the thumb between the first and second phalangeal articulations. This collar was rigidly attached by a brass rod to another collar of hard rubber which fitted loosely over the fore-arm. The outer circumference of this collar was of such a size that it fitted snugly into the mouth of the glass cylinder. The object of this device was to prevent the arm from slipping farther into the plethysmograph. To prevent the arm slipping out of the plethysmograph, the device described by Shields[3] was used. This consisted of two hinged hard rubber rings. The larger one of these rings fitted around the end of the glass cylinder, the other ring, of just sufficient size to allow the fore-arm to pass through it, was connected to the first by screw clamps, so that the smaller collar could be pressed up against the end of the cylinder, and in this way prevented the collar within the cylinder from being pulled out.

The plethysmograph was swung from the ceiling, and was so arranged that it could be adjusted to any desired level. The elbow was supported by means of a sling, which was fastened to the chain holding the plethysmograph.

The greatest difficulty encountered in all plethysmographic experiments has been to secure some device whereby the arm could be enclosed within the plethysmograph in such a manner as to prevent leakage from the cylinder, and at the same time to avoid compression of the arm. The errors which occur in either case spoil the records obtained. The original device employed by Mosso,[4] consisting of a rubber sleeve, was not entirely satisfactory when used alone, the chief objection being the difficulty in adjusting the sleeve to the size of the arm of the subject for each experiment, without causing undue compression of the arm. The rubber sleeve only serves this purpose when it is very carefully adjusted to the arm. A device has been used in these experiments which can readily be adjusted to any arm without danger of either a leakage from the instrument or a compression of the arm. A piece of heavy rubber band tubing, fifteen centimetres in length and of sufficient diameter to allow it to fit loosely around

[1] HOWELL: Journal of experimental medicine, 1897, ii, p. 313.

[2] SHIELDS: Journal of experimental medicine, 1896, i, p. 74.

[3] SHIELDS. Journal of experimental medicine, 1896, i, p. 74.

[4] MOSSO: Ueber den Kreislauf des Blutes im menschlichen Gehirn, Berlin, 1881; Die Temperatur des Gehirns, Berlin, 1894.

the fore-arm, was drawn over the hand and fore-arm, the upper end of the sleeve reaching the elbow. A surgeon's glove, of thin rubber and provided with a long sleeve, was then drawn over the hand and the heavy rubber sleeve. A second piece of band tubing, similar in every respect to the piece first slipped over the fore-arm, was then pulled over the sleeve of the rubber glove, in such a manner that the thin rubber sleeve was sandwiched between the two pieces of heavy rubber tubing. The thumb was next secured in the holder used to prevent the arm from slipping too far into the cylinder, and the hand was then thrust into the plethysmograph. The upper ends of the heavy rubber tubing, between which lay the thin rubber sleeve, were then inverted over the mouth of the cylinder, and were securely tied. The hard rubber collar was next adjusted on the arm in such a manner that the rubber sleeves were tightly clamped between the inner collar and the smaller one of the collars on the outside of the cylinder, and this collar was then firmly fastened to the hard rubber ring encircling the end of the cylinder. The rubber glove completely closed the open end of the glass cylinder, so that there was no possibility of a leak. The object of the pieces of heavy rubber band tubing, one on each side of the thin rubber sleeve, was to reinforce the thin rubber at the mouth of the plethysmograph. Were it not for this protection the water within the cylinder would affect the thin sleeve, pushing it out, and in this way the accuracy of the instrument would be destroyed. Since the rubber sleeves used did not bind the arm, there was no danger of compression, the arm being under the same pressure it would have been were it enclosed in the same volume of water without the sleeve intervening. The hand and about nine centimetres of the fore-arm were enclosed in the thin rubber sleeve; this sleeve was forced snugly against the skin by the water within the instrument, the water at the same time forcing any air out that might have been imprisoned between the glove and the arm. With this arrangement the thin rubber glove acted as a second skin, allowing the arm and hand to increase or decrease readily in volume, and these changes in the volume were promptly recorded by the corresponding outflow or inflow of water from the plethysmograph to the hanging test-tube. The adhesion of the thin glove to the arm entirely prevented any chance of air forcing its way between the glove and the skin.

When the subject was ready for an experiment, the glove was drawn on and the arm secured in the plethysmograph. The three-way stop-cock was turned so that the plethysmograph was placed in

connection with the reservoir. As the instrument filled with water, the air within the plethysmograph was forced out through the small opening in the top of the cylinder. As soon as the instrument was filled with water, this opening was closed. The water was still forced into the plethysmograph from the reservoir until the hand was under considerable pressure. This pressure was sufficient to force out any air that might have remained between the sleeve and the arm, and it was also effective in fitting the glove closely to the arm and hand. When the glove had been pressed down against the hand, the stop-cock was turned so that the plethysmograph was placed in connection with the recorder, the water supply from the reservoir being shut off at the same time. Under these conditions water flowed from the plethysmograph to the recording test-tube until the pressure within the cylinder, and consequently the pressure exerted against the arm, was equal to the level of the column of water in the test-tube. If the water level in the test-tube was higher than the level of the cylinder, the arm in the plethysmograph was subjected to positive pressure. If, on the contrary, the cylinder was higher than the level of the water in the test-tube, the arm was under negative pressure. This must be avoided, for, as was shown by Shields,[1] a positive pressure on the arm may cause a marked constriction, while, on the other hand, negative pressure causes the arm to dilate. If the test-tube was so arranged that the level of the water within it was at the height of the middle of the cylinder, then the arm within the cylinder was half of it under a slight negative, and half of it under a slight positive pressure.

Records of the blood-pressure were taken by a modification of Mosso's sphygmomanometer.[2] The apparatus consisted of two glass tubes, one above the other, and of sufficient size to allow the fingers to be easily inserted. The tubes, which were connected with each other, were filled with water, which placed the fingers under a counter pressure. To prevent leakage, the fingers were inserted into thin rubber or membrane fingers which were securely fastened to the cylinders. Besides these thin fingers, thin leather collars were pulled over the rubber fingers to reinforce them and prevent the possibility of bulging of the rubber when the fingers were subjected to great pressure. The hand was held in position by a hard rubber collar which fitted around the wrist and which was secured to the base sup-

[1] SHIELDS: Journal of experimental medicine, 1896, i, p. 74.
[2] MOSSO: Archives italiennes de biologie, 1895, xxiii, p. 177

porting the glass tubes. This prevented the fingers from slipping out of the apparatus when pressure was applied. The pressure was regulated by means of a pressure flask swung from the ceiling, and so arranged that it could be raised or lowered as was desired. The pressure was registered by a mercury manometer arranged to take graphic records.

The method of determining the blood-pressure by this instrument was as follows: the fingers were first inserted into the tubes, and the hand secured in its position. The pressure on the fingers was increased gradually by raising the pressure bottle. As the pressure was increased, the amplitude of the pulsations increased until a certain pressure was reached; any increase in the pressure beyond this point caused the pulse to diminish in amplitude, the pulse being entirely obliterated if the pressure was raised to a sufficient height. This point having been reached, any decrease in the pressure was followed by a return of the pulse and an increase in its amplitude, the maximal amplitude on decreasing the pressure being observed at about the same pressure as with the increasing pressure. The variation in the maximal amplitude of the pulsations on the rising and descending scale never amounted to more than five millimetres of mercury. If before the pressure records were taken, the pressure was rapidly raised and then lowered, the readings secured were almost the same with both ascending and descending variations in the pressure. It was found that the temperature of the water used in the instrument had a great effect upon the amplitude of the pulsations. If the water was cold, the amplitude of the pulse was very small, and it was with difficulty that the maximal pressure could be distinguished. When the water used was of a higher temperature than that of the fingers, the pulsations were markedly increased in amplitude, and the differences in the amplitude when the fingers were under different pressures were easily recognized.

In order to test the accuracy of the principle of the Mosso sphygmomanometer, experiments were made upon dogs. A small membrane tube, of sufficient size to allow the carotid artery of a dog to slip through it easily, was fastened securely to one end of a glass tube 8 cm. long and 1 cm. in diameter. The membrane tube was pushed into the glass tube and was prevented from slipping out by a cork fastened into the end of the tube. A small hole was bored through the centre of the cork, large enough to allow the artery to be passed through it. The opposite end of the glass tube was closed

by a cork through which a small glass tube entered the cylinder.
This tube was connected by a glass " T " piece to an ordinary mer-
cury manometer and to a pressure bottle. The carotid artery to
be experimented upon was exposed and carefully dissected out
from the tissues for a distance of about eight centimetres. It was
then ligated and cut through. To the peripheral stump a strong
thread was attached, and this was pulled through the cork and the
membranous tube, until at least five centimetres of the artery were
enclosed within the tube. The free end of the artery and of the
membranous tube were then tied securely together, in such a
manner that there was no leak when pressure was applied. The
artery and membrane tube enclosing it were held in position in the
glass tube by a thread tied to the free end of the artery and passed
out through the cork at the distal end of the glass tube. The appa-
ratus was next filled with water from the pressure flask. Even at
zero pressure pulsations were visible in the mercury manometer, and,
as the pressure was raised, these oscillations of the mercury became
more pronounced until a certain pressure was reached, at which
point the amplitude of the pulsations was maximal. Any increase
or diminution in the pressure from this point caused a diminution in
the amplitude of the pulsations. Two experiments were made with
this apparatus, in one of which the right and in the other the left
carotid artery was fastened in the glass tube of the sphygmoma-
nometer. The carotid artery of the opposite side was connected
in the usual way with an ordinary mercury manometer and served as
a control to the pressure observations made with the sphygmomano-
meter. The results obtained in these experiments are given below.

Experiment 1. — Length of time during which the observations were taken, 48 minutes.
Sphygmomanometer on the right carotid.

Right Carotid.	Left Carotid.
142 mm. Hg.	150 mm. Hg.
140 " "	148 " "
150 " "	152 " "
141 " "	149 " "
136 " "	144 " "
134 " "	144 " "
Average 140.5 mm. Hg	Average 147.8 mm. Hg.

In this experiment the pressure registered in the left carotid is a
few millimetres higher than the pressure registered on the right side.
In the following experiment the sphygmomanometer was placed on

the left carotid and the mercury manometer was connected with the right carotid artery. The results of this experiment are shown in the following table:

Experiment 2.— Period during which the observations were made, 1 hour and 20 minutes. Sphygmomanometer on the left carotid.

Right Carotid.	Left Carotid.
153 mm. Hg.	160 mm. Hg.
144 " "	144 " "
139 " "	143 " "
130 " "	138 " "
137 " "	143 " "
138 " "	140 " "
145 " "	150 " "
142 " "	148 " "
128 " "	132 " "
230 " "	134 " "
Average 138.6 mm. Hg.	Average 143.2 mm. Hg.

In this experiment the greatest pressure was also recorded by the instrument in the left carotid.

The temperature was registered by standard thermometers, readings being made every fifteen minutes. The rectal temperature was obtained by thrusting a thermometer up the rectum about five centimetres. The temperature was also taken of both the arms. A small mat of cotton was fastened loosely to the arm, and the thermometer was thrust between the cotton and the skin. The cotton prevented the slight variations in the room temperature from affecting the readings of the thermometer. The pulse was taken from the radial artery, the number of pulsations counted in one-half minute being doubled and the result taken as the number for one minute. The respiratory rate was obtained by simply counting the number of respirations in one minute.

The subject for the experiment was placed upon a bed. He was allowed to rest from fifteen minutes to one-half hour before any records were taken. The subject was kept as quiet as possible, and all unnecessary noise on the part of the observers was avoided. When the subject had rested a sufficient time, the readings were begun, two or three readings being taken before any suggestion was given to the subject.

DESCRIPTION OF THE PLETHYSMOGRAPHIC CURVES.

Over twenty-five experiments have been made on hypnotic sleep in the present investigation. The plethysmographic records obtained in the first few experiments were not so satisfactory as were the tracings obtained from the later experiments, owing to the difficulty encountered in so adjusting the rubber sleeve to the arm that it neither compressed the skin veins nor allowed leakage from the instrument. Although the first experiments were inaccurate in regard to the volume changes in the arm, yet the general course of the curves was the same as in the later experiments, and hence these tracings may be taken as confirmatory of the curves obtained later, in which the volume changes in the arm were more accurately registered by the recorder.

The curves shown in Figs. 1, 2, and 3 are the plethysmographic tracings of three experiments in this series, and they show the most characteristic changes noticed in a subject when in perfectly quiet hypnotic sleep.

Many investigators, among whom are Mosso,[1] Howell,[2] Shields,[3] and Kiesow,[4] have shown that, in normal physiological conditions, the arm constantly undergoes changes in its volume, and that this is true no matter what may be the position of the body. These changes are usually small, but vary greatly in amplitude, and are attributable to mental and sensory stimuli acting upon the vasomotor centres. The course of the plethysmographic curve from an individual in the normal waking state, care being taken that all muscular movement is absent, is in a general horizontal direction. On this curve there may appear rhythmic variations due to the respiratory movements and other longer, irregular, wave-like variations which probably depend upon rhythmic changes in the vasomotor centres. Besides these, the irregular variations due to sensory and mental stimulation, already referred to, are more or less abundant. With continued sensory stimulation or mental activity, the curve mounts up above the normal level, showing that there has been a decrease in the volume of the arm. In normal sleep there is

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[1] MOSSO: Ueber den Kreislauf des Blutes im menschlichen Gehirn, Berlin, 1881; Die Temperatur des Gehirns; Berlin, 1894.

[2] HOWELL: Journal of experimental medicine, 1897, ii, p. 313.

[3] SHIELDS: Journal of experimental medicine, 1896, i, p. 74.

[4] KIESOW: Archives italiennes de biologie, 1895, xxiii, p. 198.

a fall in the curve which lasts with variations as long as the sleep continues; this fall in the curve denotes an increase in the volume of the arm. In normal physiological conditions, therefore, the course of the plethysmographic curve varies under different conditions, a rise in the curve following psychical activity, while normal sleep causes the curve to sink. With this summary of the changes observed under normal physiological conditions, the phenomena observed in hypnosis may now be described in detail.

Normal Curves. — Before the hypnotic suggestion was given to the subject, the curve was allowed to establish its normal level for the recumbent position. This level having been established, suggestion was begun. The instant the suggestion of hypnotic sleep was given to the subject, there was a pronounced rise in the curve, corresponding to a constriction of the arm, the curve mounting upward for from one to ten minutes. The change in the volume of the arm during this period was by no means the same in the different experiments, varying, in round numbers, from

FIGURE 1. — Plethysmographic record of the hand and lower part of fore-arm, on the right side, during hypnotic sleep, November 28, 1890. The operator was Mr. H. M. Steele, the subject C. P. H. The subject was in good condition for an experiment. He was placed in the apparatus at 11 A. M., and rested quietly until 11.15 A. M., when quiet hypnotic sleep was suggested. He was awakened at 3.35 P. M. During the whole course of the experiment the subject was quiet, but few movements being noticed. A fall of 1 mm. in the original record corresponds to an increase in the volume of the arm of 0.576 c.c. "X" marks the moment the suggestion was begun, and the instant the subject was awakened. The curve here presented has been reduced 80 per cent.

two to eight cubic centimetres for the hand and that portion of the fore-arm within the plethysmograph. At just what point during the suggestion the subject fell into hypnotic sleep it is impossible to determine. That hypnosis does occur during this rise is probable, since the constriction of the arm corresponded, in every case, to the period during which the suggestion was given, and as soon as the suggestion was ended, the subject being in hypnotic sleep, there was a fall in the curve. In some of the experiments this fall was so small that it was easily overlooked, while in other experiments there was a marked fall indicating a change in the volume of the hand and fore-arm of at least ten cubic centimetres. This change, corresponding to a vascular dilatation of the arm, was never so rapid as the previous rise. The time during which the fall continued varied greatly in the several experiments. In some cases it lasted but one or two minutes, while in other experiments the curve continued to sink for more than two hours. The fall in the curve differed from the previous rise in another particular. The rise was as a rule continuous, while the fall was broken and often concealed for some minutes by

FIGURE 2.— Plethysmographic record of the hand and lower part of the fore-arm, on the right side, in hypnotic sleep, December 12, 1899. The operator was Mr. Steele, and the subject C. D. H. The subject was in good condition. He was placed in the apparatus at 12.10 P. M., and hypnotic sleep was suggested at 12.30 P. M. At 3.15 P. M. the subject turned his head, and at 4.45 P. M. he was awakened. With the exception of the movement noted, he remained perfectly quiet during the period of hypnotic sleep. A fall of 1 mm. in the original curve corresponds to an increase of 0.576 c.c. in the volume of the arm. "×" marks the moment suggestion was begun, and the moment the subject was awakened. The curve here presented has been reduced 80 per cent.

irregular oscillations which occurred in the path of the tracing. These variations correspond in every particular to the sharp vaso-motor variations noticed in all plethysmographic tracings in normal physiological conditions, and hence they are probably due to the same causes. After having reached its lowest point, the curve usually began to show a steady rise, which was quite gradual and continued as long as the hypnotic sleep lasted, that is, from two to five hours. The curve during this rise not only reached the normal waking level, but in every case it mounted above this level, the hand and fore-arm constricting in some cases to an extent equal to a diminution in volume of as much as thirty cubic centimetres. In a few experiments the rise in the curve was much sharper, lasting about one hour, the change in the volume of the arm being about the same as in those experiments in which the curve rose more slowly. When the curve rose in this more rapid manner, having reached its maximal height for the rapid rise, the tracing continued

FIGURE 3. — Plethysmographic record of the hand and lower part of the fore-arm, on the right side, in hypnotic sleep, December 15, 1899. The operator was Mr. Steele, the subject C. D. H. The subject had been up nearly all of the previous night, and complained of being tired when he came to the laboratory. He was placed in the apparatus at 12.10 P. M., and the suggestion of quiet hypnotic sleep was given fifteen minutes later. At 1.14 P. M. there was a great constriction of the arm. During the whole of the experiment the subject was quiet. A fall of 1 mm. in the original curve corresponds to an increase of 0.34 c.c. in the volume of the arm. "x" marks the moment the suggestion was begun, and the instant the subject was awakened. The curve here presented has been reduced 70 per cent.

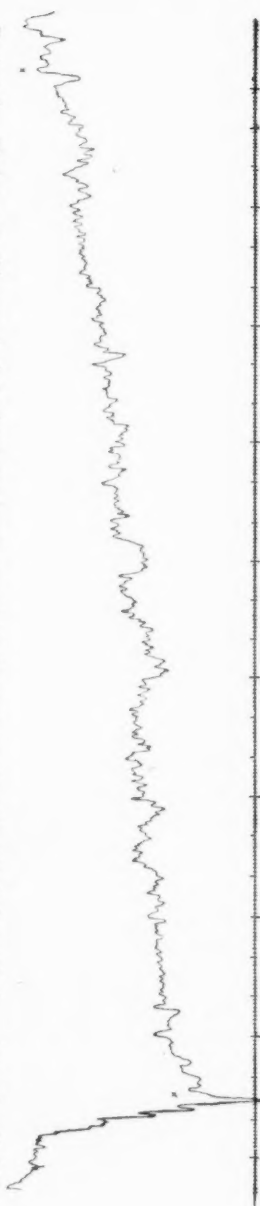to rise much more gradually, with slight oscillations, until the end of the hypnotic sleep. At the instant hypnotic sleep was ended at the suggestion of the experimenter, there was a sharp rise in the curve, lasting about one minute, similar to, and, in many cases, as great in extent as the rise which occurred at the beginning of the hypnotic suggestion. In some cases the diminution in the volume of the hand and fore-arm during this rise amounted to as much as eight centimetres. During the rise the subject usually opened his eyes and made some movements; these movements, however, did not permanently affect the curve. After reaching its maximal level, the curve began to drop, and continued to fall, broken by sharp oscillations, until the tracing had reached about the level it had at the beginning of the experiment previous to the suggestion of hypnotic sleep. The course of the curve from this time until the end of the experiment was in a general horizontal direction and corresponded in every way to the tracings of the normal waking curve. The general course of the curve of hypnotic sleep may then be described as follows: A sharp rise, lasting from one to ten minutes, is followed by a slower fall very variable in duration, usually comparatively brief, but, in exceptional cases, lasting for two hours. This fall is in turn followed by a gradual, long-lasting rise, continuing until the end of the hypnotic sleep, after which the curve sinks to the level it had previous to the suggestion. The general tendency of the curve during hypnotic sleep is upward, and this change in the record corresponds to a constriction of the hand and that portion of the fore-arm within the plethysmograph.

Besides these general changes, other secondary variations were observed. These have already been alluded to in reference to the changes noticed in normal physiological conditions, and probably depend upon the activity of the vasomotor centre. These variations were by no means uniform; in some cases a rapid rise of a few millimetres was followed immediately by an equally rapid fall, while in other cases, after a fall or a rise, the curve continued for several minutes in a horizontal direction and then gradually returned to about the former level. These changes cannot be due to external stimulation, since the subject was quiet, making no movements whatever, save those due to respiration. All noises and other forms of sensory stimuli were also excluded as carefully as possible. These oscillations are due, then, to some internal stimulus acting upon the vasomotor centre. In some of the experiments, rhythmic, wave-

like variations, such as were noticed by Howell[1] in normal sleep, have been observed; these variations were never so pronounced in the curves of hypnotic sleep as they are in the curves of normal sleep, and they were not found in all of the records of hypnotic sleep, and hence they must be considered as exceptional, depending upon the condition of the subject at the time of the experiment.

Exceptional curves. — In four of the experiments of the present series there were variations which have not been described in the plethysmographic curves of other investigators. These changes, which were of two different types, are shown in Figs. 4 and 5. In one case, Fig. 4, after gradually falling for fifty minutes, the curve suddenly mounted upward. In one such experiment there was a diminution in the volume of the hand and that portion of the fore-arm within the instrument of 16.9 cubic centimetres in three minutes, and a further

[1] HOWELL: Journal of experimental medicine, 1897, ii. p. 313.
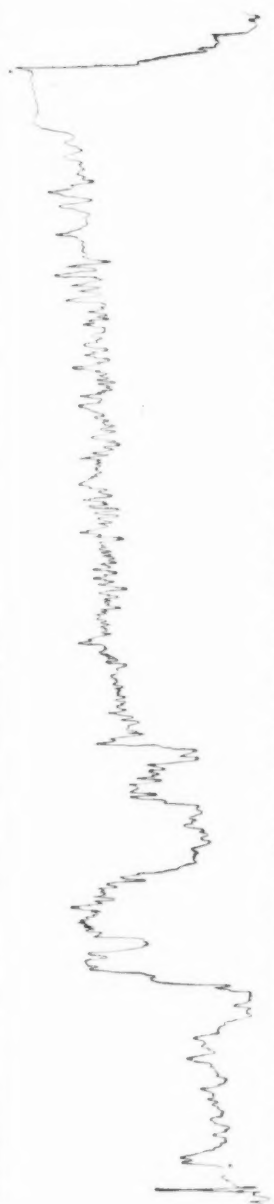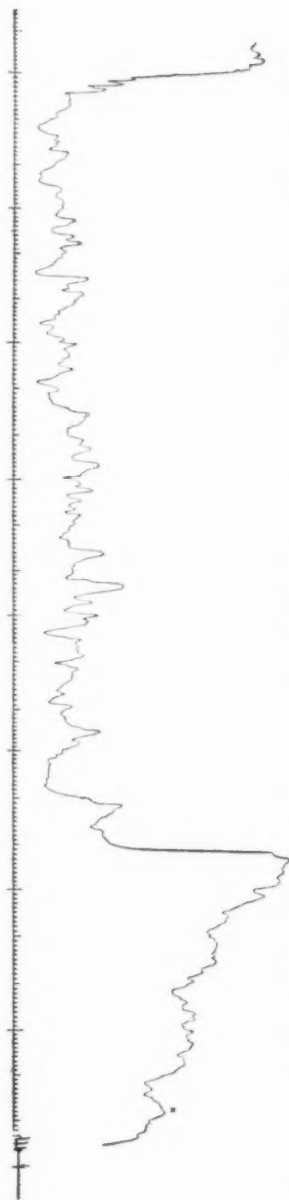

FIGURE 4. — Plethysmographic record of the hand and lower fore-arm, on the right side, in hypnotic sleep, December 21, 1899. The operator was Mr. Steele, and the subject C. D. H. The subject complained of headache when he came to the laboratory. He was placed in the apparatus at 12 M. and the suggestion of quiet hypnotic sleep was given at 12.11 P. M. During the whole of the experiment the subject rested quietly, making no apparent movements. He was awakened at 4.20 P. M. A fall of 1 mm. in the original curve corresponds to an increase of 0.33 c.c. in the volume of the arm. " X " marks the moment of waking, and the instant the subject was given a suggestion. The curve here presented has been reduced 73 per cent.
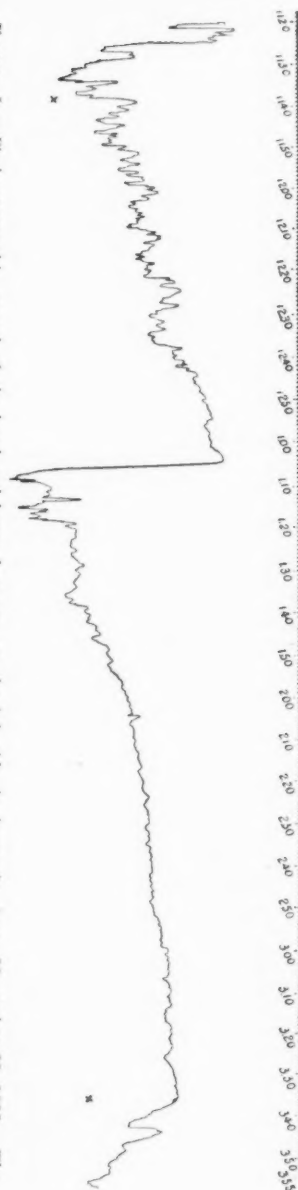
FIGURE 5. — Plethysmographic record of the hand and lower fore-arm, on the left side, in hypnotic sleep, November 29, 1899. The operator was Mr. Steele, the subject C. D. H. The subject was not in the best condition for an experiment, having been out very late the night before, and not being rested when he came to the laboratory. He was placed in the apparatus at 11.19 A. M. At 11.21 some matches which were on the table took fire. This caused a marked constriction of the arm. At 11.25 the level of the pen was changed so that it wrote 15 mm. lower than before. At 11.32 the subject moved his legs and arms, and had a violent coughing spell. At 11.36 it was suggested that the subject should pass into quiet hypnotic sleep. The subject remained quiet during the entire period of the sleep. He was awakened at 3.35. A fall of 1 mm. in the original curve corresponds to an increase of 0.35 c.c. in the volume of the arm. "×" marks the moment the suggestion was given, and the time at which the subject was awakened. The curve here presented has been reduced 79 per cent.

shrinkage of 5.6 cubic centimetres during the following fifteen minutes. This surprising constriction of the arm could not be traced to any external stimulus, nor to an alteration in the position of the hand within the plethysmograph. No difference could be noticed in the rate of the pulse or of the respiration. After this sudden rise, the tracing maintained the high level until the moment of waking, when after the usual rise which occurs on waking, the curve sank to about the level it had at the beginning of the experiment. The sudden variations already described, due to vasomotor changes, were much sharper and more numerous after this sudden rise than they were in that portion of the tracing preceding it.

In Fig. 5, a curve of an opposite character to the one just described is reproduced. After a gradual rise, lasting ninety minutes, which in this case corresponds to a diminution in the

volume of the arm of 7.6 cubic centimetres, the curve fell abruptly, the arm increasing in volume 20 cubic centimetres in seven minutes. After this sudden fall, the curve gradually rose for two hours and five minutes, rising beyond the waking level. The subject was then awakened. On waking, the tracing sank to about the level it had previous to the suggestion of hypnotic sleep. Here, again, the sharp variations of vasomotor origin differed, those preceding the fall being much sharper than the oscillations which followed it. Unfortunately the sudden fall occurred at a time when the subject was not under close observation, and the possibility of some external stimulus being one of the causes for the fall cannot be excluded. The position of the hand in the instrument could not have been altered, for had such a change occurred, the tracing would not have returned to so nearly its former level at the end of the experiment. A point of some interest in this connection is that these variations never occurred in the tracing when the subject was in the best condition for an experiment. He complained on each of these occasions of not having had sufficient sleep the night before, or at the time of the experiment he was suffering with headache.

Effect of suggestion. — A series of experiments was undertaken to determine the effect of various external stimuli upon subjects in different hypnotic conditions. It was suggested to the subject that his arm had grown smaller or that it had increased in size, or he was told that he had forgotten his name or could not open his eyes. In other cases it was suggested that the subject would hear music during his sleep, and at certain intervals a music box was set into action. The effect of suggestion during the normal waking state was always to give a sharp rise in the curve (Fig. 6) which lasted until the suggestion ended, when the tracing gradually sank to its former level. On suggesting hypnotic sleep, the phenomena observed were the same as those already described for the hypnotic sleep curves. During hypnotic sleep, the curve having risen above the normal waking level, each suggestion caused a sudden rise in the tracing of from one to five millimetres; this rise was followed by a sharp fall in the curve. The fall was closely related to the suggestion as it invariably ended with the suggestion, the curve then gradually mounted upward until it had reached about the level it had before the suggestion was given. In suggestion during hypnotic sleep there was always a fall in the curve, and in suggestion during the normal waking state there was always a rise in the tracing, no matter what the character of the
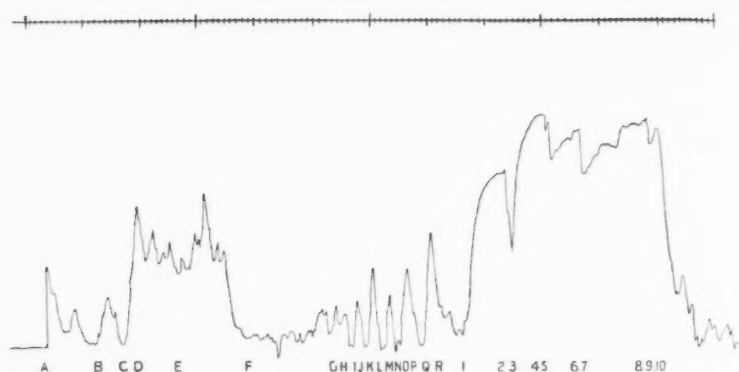
FIGURE 6. — Plethysmographic record of the hand and lower fore-arm, on the right side, taken December 7, 1899. This experiment was to determine the effect of suggestion during the normal waking and during the hypnotic sleep. Mr. Steele operator, and C. D. H. subject. A fall of 1 mm. in the original curve corresponds to an increase of 0 282 c.c. in the volume of the arm. The curve here presented has been reduced 57 per cent. The letters and figures show the points at which suggestions were given, and correspond to the following : —

A. Suggested that the subject could not open his eyes.

B. Suggested that the subject could open his eyes.

C. Suggested that the right arm of the subject was swelling.

D. Suggested that the arm had returned to its normal condition.

E. At this point the subject was left alone in the room in order to see if the curve would sink to the level it had originally.

F. Came back into the room.

G. Suggested that the subject could not open his eyes.

H. Suggested that the subject could open his eyes.

I. Suggested that the right arm of the subject was swelling. The subject at this time made a great many movements.

J. Suggested that the arm was normal.

K. Suggested that his right arm had diminished in size.

L. Suggested that his arm was normal.

M. Suggested that there was an increased flow of blood to the arm.

N. Suggested that the arm was normal.

O. Suggested that the arm was constricting.

P. Suggested that the arm was normal.

Q. Suggested that the arm was dilating.

R. Suggested that the arm was normal.

1. Quiet hypnotic sleep was suggested

2. Suggested that the arm was swelling.

3. Suggested that the arm was normal, this suggestion was followed by the suggestion of deeper hypnotic sleep.

4. Suggested that the arm was constricting.

5. Suggested that the arm was normal.

6. At this time the subject had a coughing fit.

7. Suggested deeper hypnotic sleep.

8. Suggested that the arm was swelling.

9. Suggested that the arm was normal.

10. The subject was awakened. This occurred at 2.45 P. M.

suggestion given may have been. When a suggestion was given to a subject in hypnotic sleep, he became restless and acted as he would have done if the suggestion had been given to him while he was awake. The small but sharp rise followed by the fall in the curve was very similar to the rise and subsequent fall which always occurred on waking a subject from hypnotic sleep. The slight movements made by him cannot account for the fall in the curve, since the same movements were noticed when the suggestion was given during the waking state, in which case there was a rise in the curve. This fall can be more easily explained by assuming that in hypnotic sleep the voice of the operator partially awakened the subject. The small rise which preceded the longer fall also adds weight to this assumption. The rise which followed, the suggestion having ceased, would correspond to a return of the deeper hypnotic sleep.

DESCRIPTION OF OTHER PHENOMENA.

Blood-pressure curves. — Mosso,[1] François-Franck,[2] Gley,[3] Hill,[4] Colombo,[5] and others have demonstrated by various methods that muscular and mental activity, the position of the body, the time of day, and barometric conditions may all have an effect upon the blood-pressure. It is known that during normal sleep the blood-pressure is lower than it is under similar conditions when the individual is awake. As the vasomotor phenomena accompanying hypnotic sleep are the reverse of those found in normal sleep, it was decided to determine whether there was as marked a difference in the blood-pressure. It would be but natural to expect a rise in arterial pressure as an accompanying phenomenon to the vaso-constriction of the cutaneous blood vessels, but such is not the case. In fact, it is impossible to compare the curves representing the volume changes in the arm, taken by means of the plethysmograph, with the curves registered by the sphygmomanometer. While the plethysmographic

[1] MOSSO: Archives italiennes de biologie, 1895, xxiii, p. 177.

[2] FRANÇOIS-FRANCK: Travaux du laboratoire de M. Marey, 1877, p. 273.

[3] GLEY: Exposé des données expérimentales sur les corrélations fonctionnelles chez les animaux, Paris, 1897; Étude expérimentale sur l'état du pouls carotidien pendant le travail intellectuel, Paris, 1881.

[4] HILL: Journal of physiology, 1895, xviii, p. 15; 1897–98, xxii, p. xxvi; 1898–99, xxiii, p. iv.

[5] COLOMBO: Archives italiennes de biologie, 1899, xxxi, p. 345.

curves are all of them more or less similar, the blood-pressure curves differ greatly from each other, and hence it is difficult to determine what the normal pressure curve is during hypnotic sleep. The characteristic changes in the blood-pressure can only be recognized by taking the observations secured in a number of experiments, and from them constructing a mean curve. The pressure observations taken in five experiments have been tabulated in the table given below. The observations were made at intervals of fifteen minutes during the time the experiment lasted, and they include the pressure

TABLE I.

Experiment.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Dec. 13.	60	50	50	55	60	58	56	54	50	48	50	50	54	56	56	56	58	76
Dec. 15.	80	76	72	69	77	90	93	88	90	91	93	97	96	98	95	88	87	102
Dec. 21.	93	80	100	94	94	69	91	81	75	72	79	76	96	97	96	101	102	103
Jan. 11.	96	87	93	98	98	95	97	98	99	98	95	99	—	—	—	—	—	105
Jan. 16.	70	72	77	77	71	66	68	70	72	66	68	70	72	66	91	97	99	99
Average . . .	80	73	78	78	80	76	81	78	77	71	84	84	86	83	82	82	82	97

observations taken just before the suggestion was given, and those secured after the suggestion had been finished, as well as the observations made during the period of hypnotic sleep. In the first column to the left are given the pressure readings taken just before the suggestion was given; the following sixteen columns contain the observations taken at stated intervals during the suggestion; and the last column contains the readings taken just after the subject awakened.

In order to secure the general blood-pressure curve from these experiments, the average height of the pressure at each interval was taken, and from these figures a curve was plotted. This curve is given below.

The average blood-pressure in the fingers in a horizontal position was found to be eighty millimetres of mercury. Mental and muscular activity, or sensory stimulation of any kind, increased the pressure, this increase varying from five to thirty-five millimetres of mercury according to the nature of the stimulus. In order to avoid as far as

possible all external stimuli, the subject was kept quiet, and the observers made no unnecessary noise or movements. After one or two experiments, the subject became so accustomed to the apparatus that on lying down, he soon began to show signs of sleepiness. The pressure readings were begun when the subject had reached this condition, and the height of the mercury was assumed to be equal to the arterial pressure of the individual without marked mental or sensory stimulation. Following the hypnotic suggestion, there was a fall in the arterial pressure, which, on the average, amounted to seven millimetres of mercury. After this fall, the pressure fluttered around the normal level; the tendency during the first part of the

FIGURE 7. — General curve of the blood-pressure during hypnotic sleep. The figures along the abscissa represent consecutive periods of fifteen minutes. The figures along the ordinate represent the blood-pressure in the fingers in millimetres of mercury.

experiment was for the pressure to remain below the normal level, while during the remainder of the experiment the curve was above the normal level. When the suggestion of hypnotic sleep was ended, the pressure rose rapidly, the average increase in the pressure being seventeen millimetres above the pressure at the beginning of the experiment and fifteen millimetres above the pressure just before the end of the hypnotic sleep.

It was never possible to continue the pressure observations for any length of time after the subject was awakened. On this account it was not determined how soon the pressure returned to the normal level after the suggestion was ended. That it does not return to this level as rapidly as the plethysmographic curve returns to its base line is

evident, since the experiment was never stopped until the plethysmo-
graphic curve had reached about the level it had at the beginning of
the experiment, and in every case the pressure curve was still much
above this level. The marked rise at the end of the curve was coin-
cident with slight muscular movements made by the subject at this
time, and an increased heart rate which, as will be shown, was also
very striking.

In hypnosis, therefore, the general course of the blood-pressure
curve is in a horizontal direction, the pressure varying but little from
the level it had previous to the suggestion. On waking there is a
sudden rise in the curve, which is in all probability due to the effect
of increased mental and muscular activity on the heart rate, as de-
scribed in the next section.

The pulse rate. — The general pulse rate curve was determined by
the same method that was used to obtain the general blood-pressure
curve during hypnotic sleep. The pulse rate curves in the different
experiments did not show the great differences among themselves
that were noticed in the blood-pressure curves. The general char-
acter of these curves was the same in all of the experiments, and
only in the minor variations were there any differences. The table
given below contains the observations taken in the same experiments
from which the observations of the blood-pressure were obtained.

TABLE II.

Experiment.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Dec. 12.	58	56	57	52	58	62	58	57	54	54	54	56	56	57	57	58	62	74
Dec. 15.	64	62	62	62	60	62	60	61	64	66	65	60	64	60	54	56	61	68
Dec. 21.	62	58	55	60	64	63	64	62	58	60	58	66	67	57	69	72	60	70
Jan. 11.	68	60	60	63	57	56	52	58	60	60	60	62	60	—	—	—	—	64
Jan. 16.	56	52	60	52	52	56	52	52	60	60	60	58	60	—	—	—	—	62
Average . .	62	58	59	58	58	60	57	58	59	60	59	60	61	58	60	62	61	67

From the table it will be seen that before hypnotic sleep was sug-
gested, the pulse rate was sixty-two per minute; after suggestion there
was a fall in the rate to fifty-eight per minute. During the entire
period of hypnotic sleep, the rate varied about this figure, the aver-

age rate for the entire period of hypnotic sleep being fifty-nine pulsations per minute. The tendency of the pulse curve was to rise gradually, the heart beating less frequently at the onset of hypnotic sleep, than it did after the subject had been asleep for several hours. After hypnotic sleep had lasted for about three hours, the pulse rate was nearly as high as it was just before the suggestion was given. On waking the subject, the pulse increased in frequency, the average rate for the observation just after the hypnotic sleep had ended being sixty-seven pulsations per minute. In the accompanying figure is given the plotted curve constructed from the averages given in the preceding table.
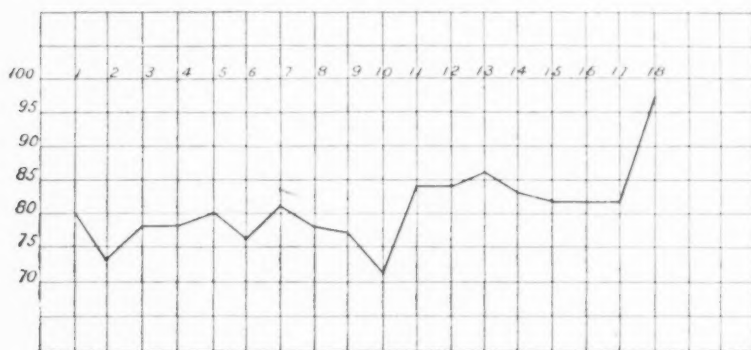

FIGURE 8. — General curve of the pulse rate during hypnotic sleep. The figures along the abscissa represent consecutive periods of fifteen minutes, those along the ordinate represent the number of heart-beats per minute.

The table and figure both show that the heart rate was slower during hypnotic sleep than during the waking state, they also show that this effect upon the heart rate was greater at the beginning of the hypnosis than it was after the hypnotic sleep had continued for a considerable time, since the rate gradually increased until at the end of four hours the heart had about the same rapidity as at the beginning of the experiment. On waking, the pulsations were more frequent than either during or before the hypnotic sleep. After waking, the rate of the pulsations did not return rapidly to the

rate previous to the suggestion, as it was always faster when the last observations were made, about fifteen minutes after the subject had been awakened, than it was at the beginning of the experiment.

Respiration. — It is well known that during periods of mental and muscular rest, as well as in sleep, the respiration is slower than during periods of activity. Since in hypnotic sleep, there are but few movements, it is but natural to expect that the respirations would be less frequent, and such is the case. Hoover and Sallman[1] have observed the changes in the respiration in a hypnotic subject who was in a hypnotic state for one week. During this period, the respiration was slower than normal, except at those times when the subject made muscular movements. At such times there was an increase in the frequency of the respirations which these authors attribute entirely to the movements. In the present experiments, the hypnotic state was much shorter than in the experiment referred to, and but few movements were noticed. In the table given below are tabulated the observations of the respiratory rate and the average rate at each reading taken in the five experiments from which the pulse and pressure observations, already described, were obtained.

TABLE III.

Experiment.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Dec. 12.	16	18	18	16	16	16	17	16	16	16	16	18	17	17	17	16	17	22
Dec. 15.	19	16	19	16	16	16	16	18	20	16	12	15	16	16	20	16	16	20
Dec. 21.	22	16	16	16	16	17	16	16	16	17	15	16	16	17	12	18	16	20
Jan. 11.	18	18	18	21	18	18	20	16	17	16	16	17	20	—	—	—	—	20
Jan. 16.	16	15	16	16	15	15	14	15	16	18	18	19	16	—	—	—	—	18
Average ..	18	17	17	17	16	16	17	16	17	17	15	17	17	17	16	17	16	20

It will be noticed that while in some of the experiments the variations were well marked, in others there was but little difference in the respiratory rate during the whole experiment. This can only be accounted for by assuming that the subject was less restless in those experiments in which the variations were but little marked than in those cases where the differences between the waking and the

[1] HOOVER and SALLMAN: Journal of experimental medicine, 1897, ii, p. 405.

hypnotic conditions were more prominent. The average figures for the experiments show that there was a fall in the respiratory rate during the period of hypnotic sleep. In the accompanying figure is shown the general course of the curve of the respiratory rate during hypnotic sleep.
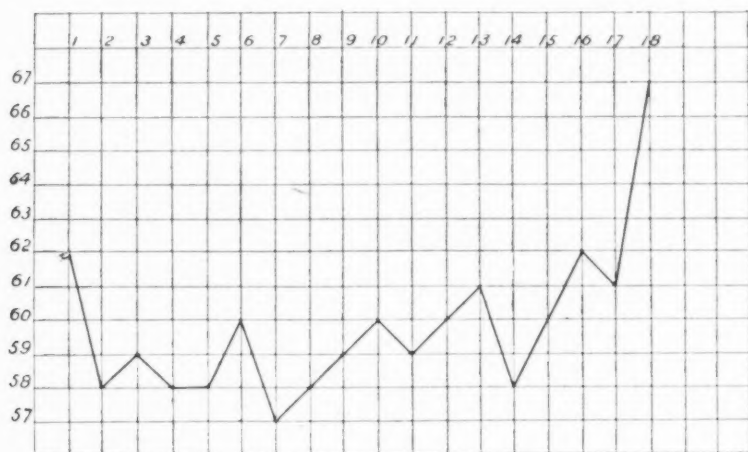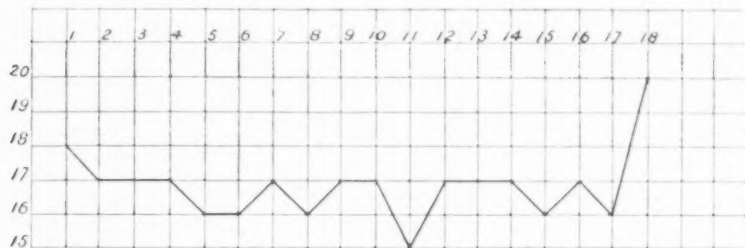

FIGURE 9. — General curve of the respiratory rate during hypnotic sleep. The figures along the abscissa represent consecutive periods of fifteen minutes, those along the ordinate represent the number of respirations per minute.

At the moment of hypnotic suggestion there was a fall in the curve; during the entire period of hypnotic sleep it ran a horizontal course with slight variations, and on waking it rapidly mounted upward, passing the level it had at the beginning of the experiment.

Temperature. — It has only been possible to obtain observations of the *rectal temperature* in two experiments. The course of the curves constructed from these observations was the same in the two experiments, and the general curve constructed from the observations may be safely assumed to represent the general course of the rectal temperature during hypnotic sleep. Table IV. contains the temperature observations taken in the two experiments and also the averages obtained from these observations, and Fig. 10 represents the curve plotted from the mean figures.

These observations and the plotted curve of the general course of the curve of the rectal temperature show that there is but little change in the rectal temperature.

The rectal temperature was always higher at the beginning of the experiment than it was at any time during the rest of the experiment. On suggesting hypnotic sleep, there was a slight fall in the temperature, and during the period of hypnotic sleep the temperature slowly and gradually fell. When the subject was awakened the temperature rose, but it did not reach the height it had before the suggestion was given.

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It may be said that the general effect of hypnotic sleep is to slightly lower the rectal temperature.

Surface Temperature. — The effect of hypnotic sleep upon the temperature of the skin was different from the effect upon the rectal temperature. The variations in the temperature were not the same in the two arms, the changes being less marked in the arm surrounded by the water in the plethysmograph than in the other arm. Table V. contains the temperature observations taken in five experiments and Fig. 11 the curves plotted from the average observations of the two arms.

The general course of the temperature curves in the two arms was the same. The general course of the curve, as shown in the figure, was as follows: On suggesting hypnotic sleep, the temperature rose rapidly, as determined by observations at intervals of fifteen minutes, this rise continuing for about one hour, after which the curve slowly and steadily sank until the end of hypnotic sleep; on awakening the subject, the curve rose rapidly, reaching a higher level than it had at any previous time during the experiment.

It will be seen that the general course of the temperature curves of the skin and rectum is not the same. While the rectal temperature remains nearly uniform, sinking slowly though slightly during hypnosis, and showing but little rise on waking, the skin temperature follows in general a reverse course to that of the plethysmographic record, rising during the first part of hypnosis, sinking during the latter portion of the sleep, and rising suddenly and markedly upon awakening.

TABLE IV.

Experiment.	Dec. 15, 1899.	Dec. 12, 1899.	Average
1	37.1	37.1	37.1
2	36.9	36.9	36.9
3	36.8	37.0	36.9
4	36.8	37.2	37.0
5	36.9	37.1	37.0
6	36.9	37.1	37.0
7	36.9	37.0	36.9
8	36.9	37.1	36.9
9	36.8	36.9	36.8
10	36.8	37.0	36.9
11	36.8	37.0	36.9
12	36.8	36.9	36.8
13	36.7	37.0	36.8
14	36.7	37.0	36.8
15	36.7	36.8	36.7
16	36.8	36.8	36.8
17	36.8	36.8	36.8
18	36.9	36.9	36.9

It should be noted, however, that the general temperature of the skin during the hypnosis was above that observed at the time the suggestion was given.

DISCUSSION OF RESULTS.

Suggested explanation of the meaning of the plethysmographic curves. — The changes which occur in the arm as the result of mental and muscular rest and activity have been shown to be due, in all probability, to vasomotor dilatation and constriction of the cutaneous blood vessels. Mosso[1] has clearly shown, in individuals with defective skulls, that there is a diminution in the volume of
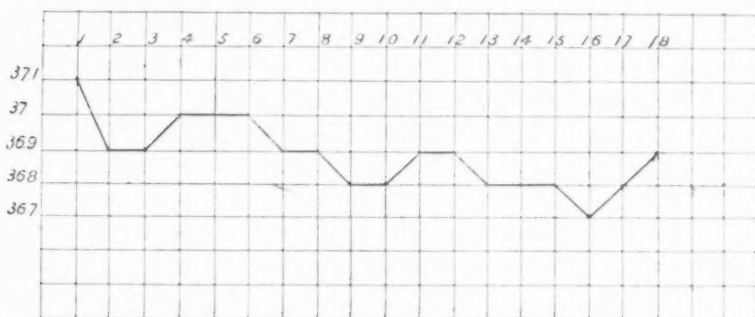
FIGURE 10. — Plotted curve of the rectal temperature observations in hypnotic sleep. Average before, 37.1°; during, 36.9°; after hypnotic sleep, 36.9°. The figures along the abscissa represent consecutive periods of fifteen minutes, those along the ordinate, the temperature in tenths of a degree centigrade.

the brain during normal sleep, and he attributes this shrinkage in the volume of the brain to a diminished amount of blood in the blood vessels of the brain. Tarchanoff[2] has shown that in sleeping dogs there is a fall in the blood-pressure, and that on waking the pressure again mounts upward. Since the vasomotor changes in the cutaneous vessels are not local, but general, this fall in the blood-pressure is easily accounted for by the diminution in peripheral resistance, by the diminished heart rate and respiratory rate. Howell[3] has observed that there is a marked increase in the size of the arm and

[1] Mosso: Ueber den Kreislauf des Blutes im menschlichen Gehirn, Berlin, 1881; Die Temperatur des Gehirns, Berlin, 1894.

[2] TARCHANOFF: Archives italiennes de biologie, 1894, xxi. p. 318.

[3] HOWELL: Journal of experimental medicine, 1897, ii. p. 313.

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TABLE V.

Right Arm.

Experiment.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Nov. 29, 1899.	33.3	33.3	33.3	33.3	33.3	33.3	33.3	32.5	32.3	32.2	32.3	32.2	32.2	32.2	32.1	32.2	32.2	32.3
Dec. 12, 1899.	32.2	32.4	32.3	32.3	32.1	32.1	32.0	32.1	32.1	32.2	32.2	32.3	32.2	32.4	32.4	32.1	32.1	34.2
Dec. 15, 1899.	33.2	33.5	33.7	34.0	34.0	33.8	33.7	34.0	33.4	33.6	33.6	33.7	33.5	33.3	33.2	33.0	33.0	33.6
Dec. 21, 1899.	32.5	32.5	32.6	32.7	33.0	33.0	33.2	33.3	33.4	33.6	33.6	33.7	33.5	33.3	34.0	34.0	34.2	35.0
Jan. 11, 1900.	33.0	32.6	32.7	33.0	33.0	33.0	33.0	32.7	32.5	32.6	32.7	32.7	33.9	34.0	34.0	34.0	35.0	33.5
Average.	32.6	32.9	32.9	33.1	33.0	33.2	33.0	32.9	32.9	32.7	32.7	32.9	32.9	32.9	32.9	32.9	32.9	33.7

Left Arm.

Experiment.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Nov. 29, 1899.	30.3	30.5	30.6	30.6	30.5	30.4	30.3	30.0	30.0	29.8	29.0	28.8	28.5	28.6	28.7	28.7	28.8	29.0
Dec. 12, 1899.	33.7	33.6	33.5	34.4	34.3	34.3	34.2	34.4	34.3	34.3	34.3	34.4	34.3	34.4	34.4	34.3	34.5	
Dec. 15, 1899.	33.5	33.5	33.7	33.8	34.0	33.8	34.0	33.9	33.7	33.5	33.4	33.3	33.2	33.0	32.7	32.5	33.8	33.0
Dec. 21, 1899.	33.0	33.4	33.5	33.5	33.8	33.8	34.0	34.3	34.3	34.5	34.5	34.6	34.8	34.8	34.8	34.9	35.0	35.2
Jan. 11, 1900.	33.2	34.0	33.8	33.8	33.7	33.7	33.7	33.5	33.3	33.0	33.2	33.4	.....	.....	.....	.....	.....	34.0
Average.	32.7	33.0	33.0	33.0	33.3	33.2	33.2	33.2	33.1	33.0	32.9	32.9	32.7	32.7	32.7	32.6	32.5	33.1

hand during normal sleep due probably to a vascular dilatation of the skin. This dilatation follows a definite course and passes off gradually toward the time of spontaneous waking.
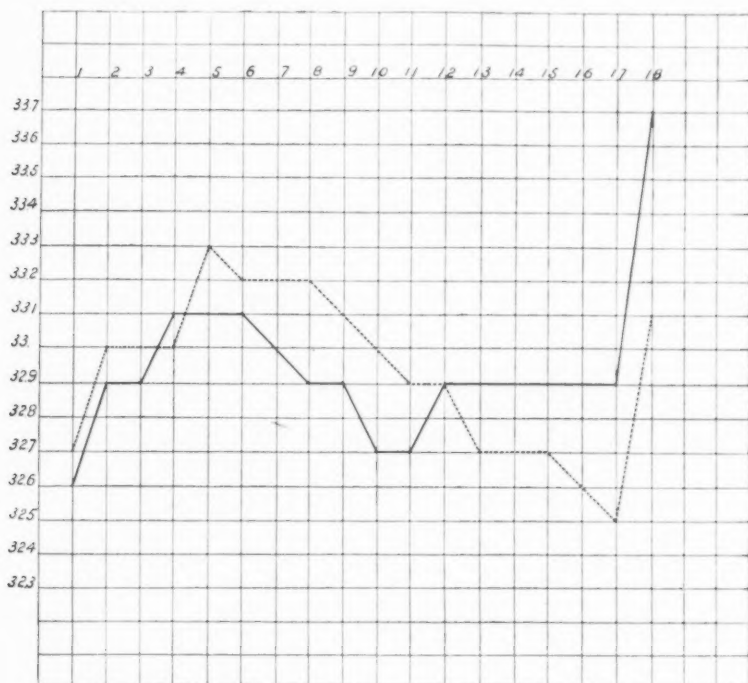
FIGURE 11. — Plotted curves of the general course of the skin temperature in the two arms during hypnotic sleep. The unbroken line represents the right arm, the broken line the left arm. The figures on the abscissa represent consecutive periods of fifteen minutes, those on the ordinate, the temperature in tenths of a degree centigrade.

In psychical activity, phenomena of an exactly opposite nature have been observed by many investigators, among whom are Mosso,[1] Kiesow,[2] Shields,[3] and Binet and Courtier.[4] These authors

[1] Mosso: Ueber den Kreislauf des Blutes im menschlichen Gehirn. Berlin, 1881; Die Temperatur des Gehirns. Berlin, 1894; Archives italiennes de biologie, 1895, xxiii. p. 177.

[2] KIESOW: Archives italiennes de biologie, 1895, xxiii, p. 198.

[3] SHIELDS: Journal of experimental medicine, 1896, i, p. 74.

[4] BINET and COURTIER: L'année psychologique, 1897, iii, p. 10.

claim that there is a peripheral vaso-constriction and a rise of blood-pressure with increased psychic activity. Gley,[1] MacDougal,[2] and Mentz[3] have observed a more rapid pulse rate under the same conditions. Binet and Courtier[4] have shown that the rise in blood-pressure differs in various conditions of mental activity, the pressure often being augmented as much as thirty millimetres of mercury. The rise in the blood-pressure in the case of mental activity is due, in part, to an increase in the tone of that portion of the vasomotor centre controlling the peripheral vessels, and in part to an increase in the pulse rate. It is evident from the results obtained by these authors that mental activity causes a constriction of the peripheral blood vessels and a rise of blood-pressure, and therefore presumably a greater flow of blood to the brain, while sleep is accompanied by a dilatation of the peripheral vessels and probably a diminished flow of blood to the brain.

Salvioli[5] claimed that a constriction of the peripheral vessels and a consequent augmentation of the blood in the brain is the characteristic phenomenon of hypnotic sleep.

Tamburini and Seppilli,[6] from plethysmographic experiments made by them, distinguish between two conditions in hypnotic sleep, namely, the lethargic and the cataleptic states. During the lethargic condition they observed that the arm dilated, while, on the contrary, in the cataleptic state there was a diminution in the volume of the arm, which they attribute to a constriction of the vessels. They further observed that the pulse was more frequent during hypnotic sleep than it was under normal conditions, but that there was no noticeable difference in this respect in the two hypnotic states. The constriction of the peripheral vessels at the moment the subject passes from the lethargic into the cataleptic state, these authors explain as the result of a vascular reflex produced by the hypnotic stimulant, either mechanical, acoustic, or visual; this reflex being

[1] GLEY: Exposé des données expérimentales sur les corrélations fonctionnelles chez les animaux, Paris, 1897: Étude expérimentale sur l'état du pouls carotidien pendant le travail intellectuel, Paris, 1881.

[2] MACDOUGAL: Psychological review, March, 1896. p. 158.

[3] MENTZ: Die Wirkung akustiker Sinnesreize auf Puls und Athmung, Philosophische Studien, 1895. xi. p. 61.

[4] BINET and COURTIER: L'année psychologique. 1897, iii. p. 10.

[5] SALVIOLI: Archivio di psichiatria e scienze penali, 1881, quoted from Tamburini and Seppilli.

[6] TAMBURINI and SEPPILLI: Archives italiennes de biologie. 1882, ii, p. 273.

analogous to that produced in normal sleep by external stimuli, which cause an afflux of blood to the brain. In these experiments the authors, so far as can be determined from their records, published in the Archives italiennes de biologie, made observations only upon the immediate effects of hypnotic sleep. Their experiments seem to have continued only a few minutes. In part, these observations agree with the experiments described in the present paper. The division of hypnotic sleep into two states, the lethargic and the cataleptic, does not seem necessary. In fact, in all of the experiments performed in this series, the plethysmographic tracings took a general course, which corresponds to the curves obtained in the cataleptic state by Tamburini and Seppilli. A fall in the curve, such as described by them for the lethargic state, was never observed in the present series, unless the gradual fall in the plethysmographic curve noticed during the first part of hypnotic sleep be considered as such. Their observations on the frequency of the pulse are the reverse of those obtained in the present series of experiments. The observations of these authors were taken during a very few minutes at most, and embraced that period during which the suggestion was being given, while the observations in the present investigation cover a period of several hours. As is well known, both mental and muscular activity exert an influence upon the rate of the heart-beat, the pulse increasing in frequency with greater mental or muscular activity. This effect is well shown by the more rapid pulse on waking a subject from hypnotic sleep, and is also apparent on waking from normal sleep. On account of the increased mental activity connected with the act of suggestion, it is but natural to expect an increase in the heart rate during this period. In the present series of experiments several minutes elapsed between the time the suggestion was given and the time at which the first observation of the heart rate was made, and the effects of the increased excitement had probably disappeared. The effect of hypnotic sleep upon the pulse rate becomes less marked as the experiment progresses, and, as has already been shown, after the sleep has lasted for several hours the rate may be as high as under normal conditions.

What do the plethysmographic curves in the present series of experiments upon hypnotic sleep indicate as to the conditions of the circulatory system? As has already been shown, the curve is somewhat complex. It may be divided into five distinct portions: 1. A sudden short rise at the time hypnotic suggestion is being given.

2. A slower, more gradual fall in the curve, following immediately the end of the suggestion. 3. A gradual, prolonged rise, lasting until the end of the hypnotic sleep. 4. A sudden rise as the subject is wakened. 5. A gradual fall in the curve, after awakening, to about the level it had at the beginning of the experiment. In order to understand the causes to which these variations are due, each portion of the curve will be discussed in turn.

1. The sudden rise at the beginning of the hypnotic sleep is similar in every particular to the rise observed under normal conditions from increased psychical activity. In the case of suggestion it is due, in all probability, to the voice of the operator acting as a sensory stimulus, and to the effort on the part of the subject to concentrate his mind upon the suggestion given. The mental stimulation gives its normal reaction, causing a vaso-constriction of the peripheral blood vessels, and probably a greater flow of blood to the brain.

2. The fall that follows this rise may be due in part to the absence of external stimuli, and in all probability to a diminished psychic activity.

3. The gradual prolonged rise in the curve which follows this fall is the striking feature of the plethysmographic record. From the observations of the pulse rate it has been shown that during hypnotic sleep there is a slower rate than during the waking condition. It has also been shown that the average blood-pressure is lower during the period of hypnotic sleep than in those portions of the experiment before and after the period of sleep. The gradual rise in the curve is therefore to be explained by a vaso-constriction in the parts observed. We may assume that this constriction affects chiefly the skin vessels, since it is known that they are well supplied with constrictor fibres, and, furthermore, that this constriction is not limited to the parts examined, but affects the skin generally, just as the opposite condition of dilatation does in normal sleep. As to the effect of this constriction on the supply of blood to the brain, we can only suppose that, following the analogy of the constriction observed in psychical activity, it would result in a steady increase in the blood flow to the brain as the constriction gradually progressed. It must be remembered, however, that unlike the condition of psychical activity, the pulse rate during this period is below normal, a factor which would tend to have an opposite effect on the circulation through the brain. The general blood-pressure, which

might be considered as representing the balance of these two opposing factors, and therefore as determining whether or not there was probably an actual increase in the blood-flow to the brain, presents an irregular course. Its characteristics are not sufficiently definite to admit of a positive conclusion in this respect, so that whether or not the peripheral constriction really results in an increased flow to the brain must remain undetermined. It may be emphasized here that while during increased psychical activity we have increased peripheral constriction and increased heart rate, during hypnotic sleep these two factors are dissociated, the peripheral constriction being increased and tending to show a heightened mental state, while the pulse rate, on the contrary, is lowered much as it is in normal sleep.

4. The sudden rise in the curve at the moment the subject is wakened is probably due to the action of sensory and mental stimuli connected with the act of awakening.

5. The large fall to the normal which occurs when the subject fully awakes is probably due to the cessation of the effects of hypnotic suggestion and a consequent passing off of the peripheral constriction, that is, to a lessening of the tone of the vasomotor centre, aided possibly by a more rapid heart rate.

Probable explanation of the changes in the blood-pressure. — The blood-pressure, as has already been stated, does not pursue a regular course during hypnotic sleep, nor do the curves obtained from the different experiments have many characteristics in common.

Hill [1] has demonstrated that in bodily rest, quiet mental work and sleep, there is a fall in the arterial pressure. This has been confirmed by Oliver,[2] Colombo,[3] and Johansson.[4] On the other hand, these investigators have observed the effect of muscular activity upon the blood-pressure, and they state that during muscular exercise there is always an elevation of the blood-pressure. With continued exercise and in muscular fatigue, the pressure does not remain at this high level, but sinks to the normal and often below the normal level.

The same general phenomena have been observed in psychical

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[1] HILL: The cerebral circulation. London, 1896.

[2] OLIVER: Journal of physiology, 1895, xviii, p. 230; 1897-98, xxii, p. li; 1898-99, xxiii, p. v.

[3] COLOMBO: Archives italiennes de biologie, 1899, xxxi, p. 345.

[4] JOHANSSON: Skandinavisches Archiv für Physiologie, 1895, v, p. 20.

activity. Strong sensory excitation elevates the pressure about ten
millimetres of mercury, and intense intellectual work augments the
pressure more than twenty millimetres, according to the experiments
of Binet and Vaschide.[1] From the experiments mentioned it is
evident that mental and muscular activity increase, while rest and
sleep tend to lower the blood-pressure.

Hill,[2] in his experiments as to the effect of gravity upon the
blood-pressure, has discussed the interaction of the two chief factors
in the regulation of the pressure, namely, the pulse rate and the
tone of the blood vessels. According to him, the blood-pressure in
the morning and in the evening is nearly the same, but the influence
of these factors in maintaining this pressure is reversed. In the
morning the pressure is kept at the normal level through alterations
in the tonicity of the blood vessels, while at night, on account of
general fatigue, the vasomotor centre is no longer able to maintain
the tone of the vessels, and only by an increased heart rate can the
pressure be kept at the former level. Stewart[3] has further shown
that the output of the heart may vary greatly with a constant heart
rate, and on the other hand, the output may be constant with a
variable pulse rate. It is evident, therefore, that the blood-pres-
sure, in normal physiological conditions, is a resultant of the com-
bined action of these two factors. With increased vaso-constriction
of the peripheral vessels and an increased heart rate there should be
a rise in the arterial pressure. This is observed in conditions of
mental and muscular activity, while in such conditions as rest and
sleep, the pulse rate being slower and the peripheral vessels dilated,
the resulting phenomenon is lowered blood-pressure.

In hypnotic sleep, as was stated in the preceding section, these
factors, *i. e.*, vascular tone and heart rate do not vary in the same
direction, but are dissociated, each having an opposite influence
upon the blood-pressure. Vaso-constriction of the skin, through
the increased resistance of the peripheral vessels, tends to raise the
general arterial pressure; while on the other hand, the slower pulse
rate lowers the pressure, the result in general being an irregular
curve. The only case in which any direct similarity can be traced
between the course of the pressure curve and those of the volume

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[1] BINET and COURTIER: L'année psychologique, 1897. iii, p. 10.

[2] HILL: Journal of physiology, 1895, xviii, p. 15; 1897–98, xxii, p. xxvi;
1898-99, xxiii, p. iv: The cerebral circulation. London, 1896.

[3] STEWART: Journal of physiology, 1897–98, xxii, p. 159.

changes in the arm and changes in the heart rate, was in the experiment of December 12, 1899, in which the general course of the pulse rate and the blood-pressure curves was the same. If the changes in the effect of the heart rate and the vascular tone had been constant, the blood-pressure would have been expressed by the algebraical sum of the two. As is shown by a comparison of the general pressure curve with the curve of the heart rate and with the plethysmographic tracings, the course of the pressure curve, although taking the general direction it should have taken if the influence of these factors had been constant, contains many irregularities which are due, some to the heart rate and some to the vascular tone, as the influence of one or the other of these factors prevails. A similar relation is evident from a study of the curves for each experiment.

Provided the assumption is correct that during the period in which the hypnotic suggestion is being given there is increased mental activity, there should be a rise in the blood-pressure at this time. It cannot be definitely stated that such is the case, since no determinations of the pressure were made during this period. In every case, with the exception of the experiment of December 15, 1899, a fall in the pressure was observed when the first reading for the period of hypnotic sleep was made. As the pressure tracing was not continuous, the readings being taken but once in fifteen minutes, this first observation occurred several minutes after the suggestion had been given, and hence the pressure may have been augmented during this period without showing any effect upon the first pressure observation taken during the hypnotic sleep. The fall noticed at this time corresponds to the vascular dilatation and to the slower pulse rate occurring during the first part of hypnotic sleep. The course of the plethysmographic tracing and of the curve of the heart rate, during hypnosis, is at first a slow fall, followed by a steady and gradual rise until the end of the sleep. This explains in all probability the general line of the blood-pressure curve, since it follows the same course. The great variations noticed on the pressure curve are possibly due to the fact that while the vasomotor constriction is practically constant and continually increasing, the heart rate remains below the normal and varies to some extent irregularly; the pressure might, therefore, show considerable fluctuation, according as these factors co-operated or varied in opposite directions.

In hypnotic sleep, therefore, the blood-pressure depends upon both the vaso-constriction of the peripheral vessels and upon the pulse rate, the pressure being above or below the normal blood-pressure, according as the vascular tone of the peripheral vessels or the heart rate has the greater influence.

SUMMARY AND GENERAL CONCLUSIONS.

The facts observed during this investigation of hypnotic sleep and the explanations suggested may be summarized as follows:

1. On suggesting hypnotic sleep there is a sudden, comparatively short-lasting, constriction of the arm, the plethysmographic tracing mounting steadily upward until the act of suggestion is ended. The diminution in the volume of the arm is probably due to the vaso-constriction of the peripheral vessels of the arm, the reaction being similar to that known to be produced normally by sensory stimuli leading to increased psychical activity.

2. When the act of suggestion ends there is a fall in the plethys-mographic tracing. The fall differs in extent and in duration in the different experiments, varying, in round numbers, from one to thirty millimetres, which corresponds to a volume change in the hand and that portion of the fore-arm within the plethysmograph of from two-tenths to ten cubic centimetres and in duration, from one minute to two hours. This fall in the curve is not continuous as is the rise, but is broken by minor variations, in all probability due to vasomotor excitation.

3. The curve, after falling for a variable length of time, gradually and steadily rises during several hours, and continues to rise until the end of the hypnotic sleep. This portion of the curve is irregularly interrupted by minor oscillations due to variations in vascular tone. The diminution in the volume of the hand and that portion of the fore-arm within the plethysmograph in many of the experiments amounted to thirty cubic centimetres. The rise is undoubtedly due to a vaso-constriction of the peripheral vessels.

4. At the instant the sleeper is awakened there is a sudden, but brief, rise in the curve, corresponding to a greater constriction of the peripheral vessels; this constriction often amounted to a change in the volume of the hand and fore-arm of eight cubic centimetres. This change in all probability is due to the action of mental and sensory stimuli upon the vasomotor centre during the act of awaken-

ing. After this brief rise, caused by the stimulus of awakening, the curve rapidly falls until the tracing has reached about the level it had before the suggestion of hypnotic sleep was given.

5. Immediately following the act of suggestion, there is a fall in the arterial blood-pressure as measured in the fingers, which on the average amounts to seven millimetres of mercury. During the period of hypnotic sleep the pressure flutters about the normal level; the tendency during the first part of the sleep is for the pressure to remain below the normal, and during the remainder of the experiment to gradually increase, often rising above the normal level. On waking, there is always a sudden rise in the pressure, the pressure increasing as much as fifteen millimetres of mercury. The gradual rise in the pressure toward the end of the period of hypnotic sleep is, on the whole, parallel with the increased vaso-constriction of the peripheral vessels and the increasing pulse observed during the same period.

6. The pulse rate is slower during hypnotic sleep than before or after that period. The heart beats less rapidly during the first part of hypnotic sleep than later in the period. On waking the pulse is accelerated, the heart beating more rapidly than at any other time during the experiment. This more rapid pulse rate continues for a considerable period, from fifteen to twenty minutes, after the subject is entirely awake.

7. During hypnosis the respiratory rate is slower than during the normal waking state. The respiration is slower during the first portion of hypnotic sleep than later in the period. When the sleep continues for several hours, the respirations may be even more rapid than they were before the suggestion was given. On waking the subject, the respiratory rate is increased, the rate being more rapid than at any other time during the experiment.

8. There is a steady although a very slight fall in the rectal temperature during hypnotic sleep. The greatest change in the rectal temperature recorded in an experiment was but four-tenths of one degree. On waking, the rectal temperature rises slightly, but it does not return to the height it had previous to the suggestion.

9. The surface temperature of the arms is higher during hypnotic sleep than at the beginning of the experiment. The temperature gradually rises during the first hour of hypnotic sleep, this increase amounting to about six-tenths of one degree. This rise is followed by a slower and more gradual fall, which lasts until the end of the hypnotic sleep and amounts to about eight-tenths of one degree.

On waking, the temperature rapidly increases, reaching a point much higher than at any other time during the experiment.

10. On giving an hypnotic suggestion to a subject during the waking state, there is a rise in the plethysmographic tracing, the rise lasting as long as the suggestion continues. As soon as the suggestion is ended, the tracing sinks to about the level it had previous to the suggestion, the effect corresponding to that of an ordinary mental stimulation. The fall at the end of the suggestion is not so rapid as the previous rise. If during hypnotic sleep a suggestion is given, the effect upon the curve is different. At the moment the suggestion is begun, there is a sharp rise in the curve; this rise amounts to but one or two millimetres and is only momentary. The curve then sinks rapidly until the end of the suggestion, after which it rises to about the level it had before the suggestion was given. The fall in the curve, as the result of the suggestion, amounts in round numbers to about ten millimetres, which corresponds to a change in the volume of the hand and that portion of the fore-arm within the plethysmograph of three cubic centimetres. The fall in the curve as the result of suggestion during hypnotic sleep is in all probability due to a partial awakening of the subject, since the small rise and the subsequent fall noticed in these cases are the same as those observed when the subject is awakened from hypnotic sleep. The rise occurring at the end of the suggestion is probably due to the return of the deeper hypnotic condition.

It is difficult to draw any general conclusions as to the bearing of these facts upon the theories of the cause of hypnotic sleep. The pronounced and increasing vaso-constriction in the arm during most of the period of sleep, for the time at least that these experiments lasted, is perhaps the most positive and suggestive result obtained. It is in general the reverse of the condition observed in natural sleep.

In the latter condition the peripheral dilatation, the slower heart beat, and the lower general blood-pressure make it probable that less blood flows to the brain. In hypnotic sleep the peripheral constriction might suggest the reverse, namely, a greater flow of blood to the brain. However this conclusion is not entirely supported by the observations on blood-pressure. This latter varied irregularly, according to the observations made, and although its general curve may be considered as substantially parallel to the volume changes in the arm, nevertheless its absolute value was not materially increased above the normal before the experiment began. The

difficulty here seems to have been that whereas in normal sleep the peripheral dilatation and lower pulse rate co-operate to lower general blood-pressure, in the hypnotic sleep the peripheral constriction is antagonized, so far as its effect on blood-pressure is concerned, by the lower heart rate. One general conclusion seems to be permissible. Assuming, as there is good reason for doing, that lessening of mental activity is accompanied by a peripheral dilatation of the blood vessels, particularly perhaps in the skin, and that increased psychical activity is accompanied by the reverse condition of a peripheral constriction, then the remarkable general tendency to a peripheral constriction during hypnotic sleep, except for a variable initial period, would point to a steadily acting mental stimulation during the time that the suggestion is effective.
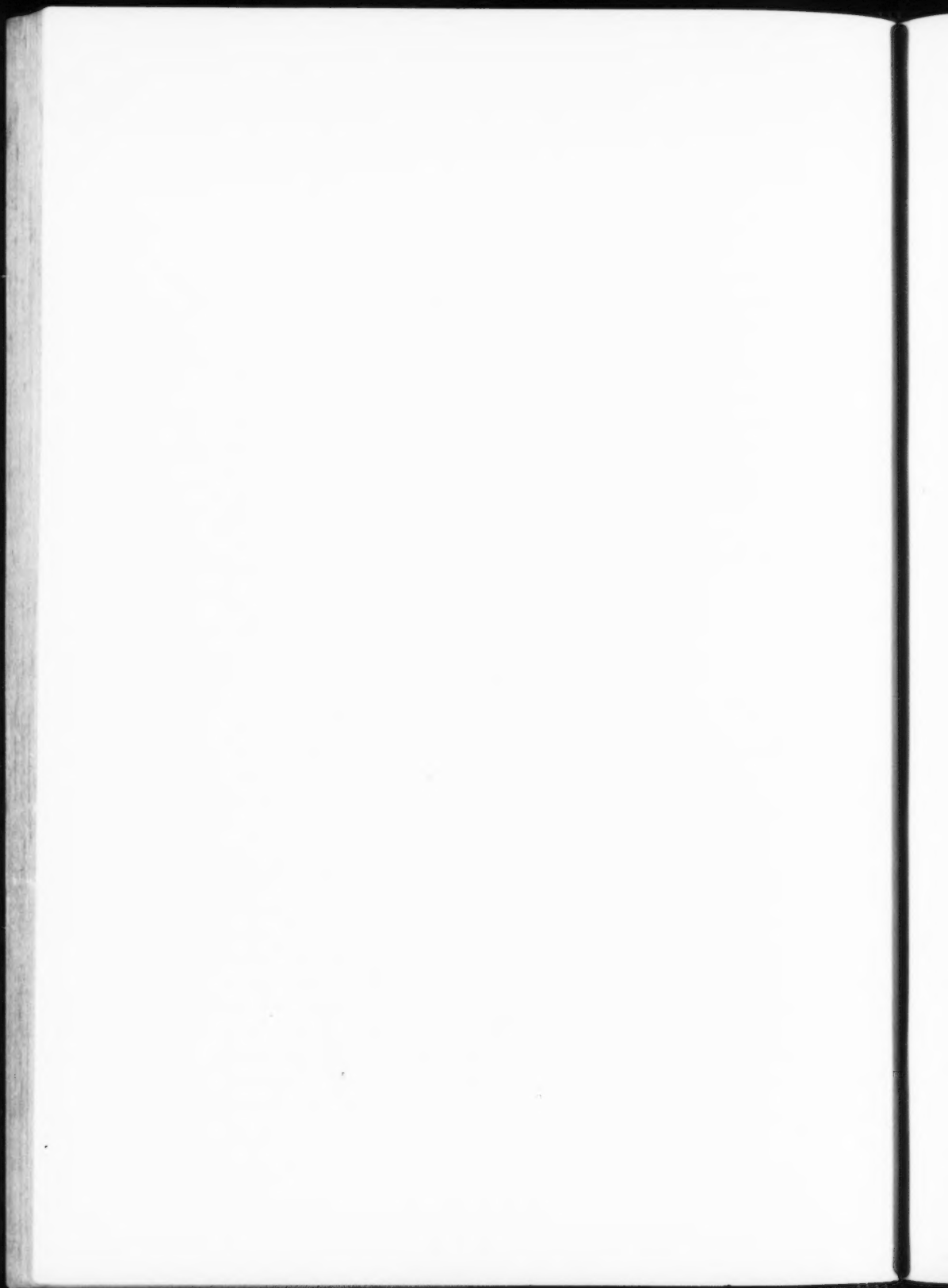
ON URIC ACID FORMATION AFTER SPLENECTOMY.[1]

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INTRODUCTORY.

IT is to-day a well accepted fact that uric acid formation occurs in mammalia under conditions quite different from those pertaining in birds and reptiles. The liver, which plays so important a rôle in the uric acid synthesis of the latter animals, is not the organ of chief importance for the similar process in the mammal. The experimental evidence upon which this position is based is diverse. Thus after exclusion of the liver from the circulation by means of Eck's fistula and ligation of the hepatic artery there is, if anything, an increase in the output of uric acid.[2] Again, in cirrhosis and hypertrophy of the liver, as well as in artificial degeneration of hepatic tissue,[3] no marked decrease in uric acid excretion has been noted. Such results would scarcely be expected if the liver were actively concerned in uric acid synthesis. In searching for an organ to which the production of uric acid might be delegated, many physiologists have turned their attention to the spleen. Thus Neumeister states: "The spleen stands in close relationship to uric acid formation, as is evident from experiments on animals and from pathological observations. This function is simply explained by the richness of the spleen in leucocytes and therefore also in cell nucleins, from the decomposition products — the nuclein bases — of which uric acid seems to arise, at least in the mammalia."[4] Hammarsten writes: "An increase in the quantity of uric acid eliminated has been observed by many investigators in lineal leucæmia, while the reverse has been observed under the influence of quinine in large doses,

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[1] A report of some of our experiments was communicated to the American Physiological Society at the December meeting, 1899. This journal, 1900, iii, p. i.

[2] HAHN and NENCKI: Archives des sciences biologiques de St. Pétersbourg, 1892, i, p. 401; DE FILIPPI: Archives italiennes de biologie. 1899, xxxi, p. 211.

[3] LIEBLEIN: Archiv für experimentelle Pathologie und Pharmakologie. 1894, xxxiii, p. 318.

[4] NEUMEISTER: Lehrbuch der physiologischen Chemie, 1897, p. 512.

which produces an enlargement of the spleen. We have here a rather positive proof that there is a close relationship between the spleen and the formation of uric acid." Again, "a direct relationship between the spleen and the formation of uric acid in man, has been sought by several investigators. According to the investigations of Horbaczewski this relationship seems to be of an indirect kind, as it probably stands in close connection with the importance of the spleen to the formation of the leucocytes." [1]

These statements, to which those of other writers [2] might be added, are for the most part due to the influence which the well-known investigations of Horbaczewski have exerted. This investigator observed that when spleen pulp is exposed to incipient putrefaction, considerable quantities of xanthin and hypoxanthin can be isolated from the mixture. If, however, the pulp at this stage is subjected to conditions which facilitate oxidation, e.g., shaking with air in the presence of blood, xanthin bases are no longer obtained, but uric acid is found in place of them. These observations have repeatedly been verified. Spitzer,[3] in particular, has studied the conditions under which this mode of formation of uric acid may be facilitated. His investigations lead to the conclusion that extracts of both spleen and liver, as well as the tissue pulp, may yield uric acid when subjected to a current of air for some time, even when putrefaction is absolutely excluded. This reaction was found to be characteristic for the two organs mentioned, namely, the liver and spleen, and could not be obtained with the kidney, pancreas, thymus or blood. Furthermore, Spitzer ascertained that pure hypoxanthin and xanthin are transformed into uric acid by the oxygen of the air, when they are dissolved in extracts of the liver and spleen. Adenin and guanin may apparently undergo the same transformation, though to a far smaller extent. Spitzer concludes that the peculiar behavior of the spleen and liver, as contrasted with the other organs enumerated, may be interpreted to indicate that "even during life these two

[1] HAMMARSTEN: Textbook of physiological chemistry, translated by Mandel, 1900, pp. 200, 431.

[2] *E. g.* STADTHAGEN: Archiv für pathologische Anatomie, 1887, cix, p. 403: BUNGE: Lehrbuch der physiologischen und pathologischen Chemie, 1894, p. 314: HOWELL: American textbook of physiology, 1896, p. 273; SIMON: Manual of clinical diagnosis, 1897, p. 349; SCHREIBER: Ueber die Harnsäure unter physiologischen und pathologischen Bedingungen, Stuttgart, 1899, p. 90.

[3] SPITZER: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 192.

organs are the principal seats of uric acid formation." The author adds, however: " it must be remembered that observations made on dead material cannot be assigned to the living cells without reserve. We can therefore by no means deny the power of the last mentioned organs (pancreas, kidney, thymus, etc.) to form uric acid during life, although they perhaps possess the capacity in slighter degree than the liver and spleen." [1]

We have studied the importance of the spleen for uric acid formation in the living organism by a more direct method, namely, experiments on splenectomized animals. At the time that our investigation was begun we were unaware of the observation of Lo Monaco [2] on uric acid excretion in a man after extirpation of the spleen. He found the output after the operation approximately normal on a mixed diet; in any case, it was not noticeably diminished. The recorded observations on uric acid formation in hypertrophy and other abnormal conditions of the spleen are uncertain and in part contradictory.[3] They thus afford no definite answer to the problem.

EXPERIMENTAL.

The animals used were the dog and the cat. In removing the spleen the suggestions of Laudenbach [4] were usually followed, and the animals all made a very rapid recovery from the operation. Since the character of the diet is now recognized to be of fundamental importance in uric acid production, our feeding experiments were primarily directed towards ascertaining the influence of those foods which are known to be uric acid precursors, namely, the nucleins. *No diminution in uric acid production was observed in any case after splenectomy.* The uric acid output was observed during hunger and on a diet of casein, and subsequently the influence of uric acid forming food was noted. For this purpose we fed sheep's pancreas, which experience in this laboratory has demonstrated to occasion marked uric acid excretion. The characteristic excretion of allantoin

[1] SPITZER: *Loc. cit.,* p. 200.

[2] Lo MONACO: Bulletino della società Lancisiani degli ospedali di Roma, 1894, xiv, p. 102; also Schmidt's Jahrbücher, 1896, cclii, p. 109.

[3] Cf. for example, STADTHAGEN: Archiv für pathologische Anatomie, 1887, cix, p. 390; THOMAS: Neubauer and Vogel's Analyse des Harns, 1890, p. 241; SIMON: Manual of clinical diagnosis, 1897, p. 349. SCHREIBER, *loc. cit.,* 1899, p. 91.

[4] LAUDENBACH: Archives de physiologie, 1896, p. 693.

first noted after pancreas feeding by Salkowski[1] in the dog, and by one of us in the case of the cat,[2] was likewise always observed. Thus, from the urine of a spleenless dog to which $1\frac{1}{4}$ kilos of fresh sheep's pancreas were fed in three days, no less than 0.85 gram of allantoin crystallized out on concentration. A small spleenless cat fed with one kilo of fresh sheep's pancreas in five days, yielded 0.65 gram of allantoin in a similar manner. We have had occasion to feed lymphatic glands, such as are frequently found abundant throughout the pancreatic tissue of sheep and in the submaxillary region of the ox. In each case a large rise in the uric acid output has been noted in the case of both normal and spleenless animals; the yield of allantoin was noticeably large. So far as we are aware, these observations are new and give further indication of the importance of glandular tissue of this type in uric acid production.

Protocols of the feeding experiments with splenectomized animals are given below; the uric acid was determined by the Ludwig-Salkowski method.

TABLE I.

Medium sized dog. The casein fed was freshly precipitated from skimmed milk and squeezed as dry as possible. The pancreas was obtained from sheep, and was freed from fat and sterilized. Water was freely allowed. The urine was not removed by catheterization, hence daily averages from three day periods are given.

Day.	Food. grams.		Urine.	
			Vol. c.c.	Uric Acid. mgr.
1	Casein,	200.	110	26 ⎤ (Average)
2	"		145	26 ⎬ 25
3	"		120	23 ⎦
4	Pancreas,	375.	185	115 ⎤
5	"	400.	135	78 ⎬ 76
6	"	300.	75	36 ⎦
7	None.		145	105 ⎤
8	"		55	⎬ 22 ⎬ 49
9	"		175	⎦

[1] SALKOWSKI: Centralblatt für die medicinische Wissenschaften, 1898, p. 929.
[2] MENDEL and BROWN: This journal, 1900, iii, p. 261.

TABLE II.

Medium sized dog. Pancreas prepared as in preceding experiment. Water *ad libitum*.

Day.	Food. grams.	Urine.	
		Vol. c.c.	Uric Acid. mgr.
1	None.	54	15 ⎤ (Average)
2	"	50	15 ⎬ 14
3	"	52	12 ⎦
4	Pancreas, 345	225	125 ⎤
5	" 360	170	96 ⎬ 119
6	" 530	130	138 ⎦

TABLE III.

Cat. The animal had previously been fed on casein for several days. The pancreas was prepared as above.

Day.	Food. grams.	Urine.	
		Vol. c.c.	Uric Acid. mgr.
1	Pancreas, 65	25 ⎤	
2	" 120	50 ⎬ 37	
3	" 100	65 ⎦	
4	None.	35 ⎤	
5	"	20 ⎬ 2	
6	"	18 ⎦	

The following protocol is added to demonstrate uric acid excretion during hepatic degeneration in a spleenless dog. The results are of interest because they illustrate the production of uric acid after exclusion of the function of the two chief organs to which this pro-

cess has been attributed.   The action on the liver was brought about by subcutaneous injection of oleum phosphoratum.   The presence of much bile pigment in the urine as well as the characteristic metabolic changes before death gave evidence of the hepatic action of the poison.   Histological examination of the liver cells also revealed pathological changes in that organ.   The dog had already received four injections of phosphorus oil during the twelve days preceding those here recorded.

TABLE IV.

Spleenless dog.   Phosphorus poisoning.

Day.	Urine.			Remarks.
	Vol. c.c.	Uric Acid. mgr.	Total N. grams.	
1	245	77	8.54	No food.
2	350	103	7.31	" "
3	290	163	9.01	" " ½ c.c. oleum phosphoratum, subcutaneously.
4	140	118	7.06	" " 1
5	180	120	7.58	" " 1
6	163	100	6.78	" " 1
7	158	126	6.12	150 grams **pancreas** fed.
8	260	171	9.06	50 grams desiccated **thymus** fed. [2]
9	184	106	6.91	25 " " " "
10	148	52	3.83	25 " " " "
11	88	37	1.60	No food. Dog very weak.
12				Dog died.

[1] Uric acid was fed in capsules on these days for another purpose; most of it was again recovered in the fæces, and the absence of any corresponding rise in nitrogen output indicates that it was not absorbed.

[2] The dog vomited some of this food.

CONCLUSION.

Our experiments demonstrate that the spleen is by no means the chief organ involved in uric acid production in the living body, if indeed it normally plays any part whatever in this process. After the exclusion of the liver and the spleen it is natural to turn to other forms of lymphoid tissue, and the lymphatic glands are at once suggested. It might be supposed that after splenectomy these glands take up the work of the spleen. Enlargement of the lymphatic glands has been recorded after removal of the spleen in man. But the very recent investigations of Vincent,[1] made to ascertain this point in the dog, fail to bring to light any permanent hypertrophy of the lymphatic glands after splenectomy. It seems improbable, therefore, that the formation of uric acid in the mammalia can be assigned at present to any definite organ, or groups of organs.

[1] VINCENT: Journal of physiology, 1900. xxv, p. ii.

ON THE PHOSPHORUS CONTENT OF THE PARANUCLEIN FROM CASEIN.

By HOLMES C. JACKSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

WHEN casein is treated with pepsin-hydrochloric acid, there usually results an insoluble substance which has received attention from a number of investigators. Lubavin[1] presented the first careful study of this product, which had previously been described by Meissner under the name of dyspeptone. He pointed out that the substance contained organic phosphorus to the extent of 4.6 per cent, the latter varying according to the conditions under which the digestive experiments were carried out.

In 1888 Chittenden,[2] while engaged in the study of the digestive products of casein, carried out a series of analyses of the insoluble residue corresponding to Lubavin's dyspeptone. His preparations contained an average content of phosphorus amounting to 2.57 per cent. It was pointed out, however, that in every case the phosphorus found in the ash of the products analyzed was as great as the total phosphorus observed and Chittenden arrived at the conclusion " that instead of being a phosphorized compound, it (dyspeptone) apparently contains no phosphorus whatsoever, other than that combined with calcium." Particularly noticeable in all his preparations is the large percentage of ash varying from 12.4 per cent to as high as 15.4 per cent. On the other hand, it may be pointed out that there is a rather striking constancy in the quantity of phosphorus found in these preparations, the significance of which will be referred to later. Chittenden was furthermore unable to reduce materially the amount of ash by any process of purification.

Since these experiments, the study of the so-called dyspeptone has been resumed by various investigators. Thus Szontagh[3] simi-

[1] LUBAVIN: Hoppe-Seyler's medicinisch-chemische Untersuchungen. 1871. iv. p. 463.

[2] CHITTENDEN: Studies in physiological chemistry. Yale University. 1889. iii. p. 66.

[3] SZONTAGH: Jahresbericht für Thierchemie. 1892. xxii. p. 170.

larly prepared a dyspeptone which he found to contain organic phosphorus to the extent of 2.96–3.53 per cent — an amount somewhat less than that observed by Lubavin. Again results of analyses by C. Willdenow [1] show a phosphorus content (3.85 per cent) agreeing more closely with the figures of Lubavin.

Salkowski,[2] in a later communication, pointed out that the amount of dyspeptone, henceforth called paranuclein in accord with the more recent nomenclature, varies according to the conditions of proteolysis. After a vigorous digestion as little as 6.7 per cent of the original casein remained undissolved as paranuclein, while under less favorable conditions the separation of paranuclein took place to the extent of even 20 per cent. Still more recently Salkowski[3] has observed that where the proteolysis is carried out under extremely favorable conditions, the separation of paranuclein may be wanting entirely. In the former cases, however, the phosphorus content of the paranuclein varied from 2.11 to 2.41 per cent — the higher content coming from products resulting from the more favorable conditions. Salkowski has found the phosphorus just described to exist in organic combination and has shown that as much as 15 per cent of the original phosphorus of the casein may be retained in the paranuclein formed from it.

From analyses of paranuclein (pseudonuclein) made by Sebelien [4] this author finds a phosphorus content varying from 2.5 to 2.76 per cent. He attributes the lack of agreement in the phosphorus content of his various preparations to variations in the extent of proteolysis. Similar results regarding the presence of organic phosphorus in the paranuclein and the effect upon its composition due to changed conditions of digestion have since been made in this laboratory by Dr. J. H. M. Knox.[5] His preparations with an ash content of 2.8 per cent, showed a content of phosphorus equal to 2.98 per cent attributable to organic phosphorus. The paranuclein obtained by gastric digestion of casein from goat's milk gave a content of organic phosphorus (2.7 per cent) agreeing closely with that from cow's milk.

[1] WILLDENOW: Inaugural-Dissertation, Bern, 1893 (Drechsel's laboratory).

[2] SALKOWSKI and HAHN: Archiv für die gesammte Physiologie, 1894, lix, p. 225.

[3] SALKOWSKI: Centralblatt für die medicinische Wissenschaften, 1893, xxxi, p. 385. *Cf.* also ALEXANDER: Zeitschrift für physiologische Chemie, 1898, xxv, p. 425

[4] SEBELIEN: Zeitschrift für physiologische Chemie, 1895, xx, p. 44/

[5] These results have not yet been published.

A review of the preceding details indicates that most authors have found a considerable content of *organic* phosphorus in this peculiar cleavage product of casein. The results of Chittenden alone stand in contrast to the others. The data of the various investigators are, however, by no means in accord with one another. They vary very widely according to the conditions under which the experiments have been carried out, both with respect to the quantity of para-nuclein formed and the composition of the latter. Furthermore, the large ash content revealed by the analyses of previous investigators makes it clear that they were working with preparations which could scarcely lay claim to any considerable degree of purity. The discrepancies noted and the more recent advances made in the field indicated, have rendered it desirable to repeat some of the older experiments and especially to ascertain the possibility of the formation of paranuclein, entirely devoid of organic phosphorus as it was assumed by Chittenden to have been formed in his experiments.

DIGESTION I.

In this first experiment, an attempt was made to repeat closely the preparation of paranuclein as outlined by Chittenden.[1] Casein prepared according to the method of Hammarsten, was thoroughly extracted with ether and dried at 105° C. for analysis. The following results indicate the purity of the product:

	N. %	P. %
Hammarsten	15.65	0.847
Jackson	15.53	0.852

Casein from fifteen quarts of skimmed milk was placed in seven litres of 0.4 per cent hydrochloric acid and warmed at 38° C. To this was added a dialyzed pepsin solution containing 0.75 grm. of commercial scale pepsin (1 : 3000). This mixture was allowed to digest for two days at 38° C., at the end of which time the undissolved residue was filtered off and washed thoroughly with water. The undissolved matter was again placed in three litres of 0.4 per cent hydrochloric acid containing 100 c.c. of a 1 per cent dialyzed pepsin solution, and allowed to digest at 38° C. for four days. Filtered from this digestive mixture, the resulting residue was treated again with a similar pepsin-hydrochloric acid solution and the proteolysis contin-

[1] CHITTENDEN: *Loc. cit.*

ued for seven days at 38° C. As the quantity of insoluble matter did not appear to decrease, it was filtered from the acid fluid and washed with water until the washings gave only a faint test for chlorides.

It seemed possible that the apparent absence of organic phosphorus in Chittenden's preparations might be due to the action of the alkali which had been used in redissolving and purifying the paranuclein. In order to determine this point, the paranuclein obtained in the above digestion was divided into two portions, each of which was subjected to the treatment outlined below. Furthermore, in order to preclude the possibility of the formation of a large ash content by the process of dialysis and precipitation employed by Chittenden, the former process was omitted in preparation **B**.

Preparation A. — Paranuclein was dissolved in 1 per cent sodium carbonate solution and filtered, thus removing the larger part of the fat left adherent to the residue of digestion. Upon addition of dilute hydrochloric acid to the alkaline filtrate, the paranuclein was thrown down as a heavy flocculent precipitate. The latter was filtered, washed and again dissolved in 1 per cent sodium carbonate solution. This fluid, after exact neutralization with dilute hydrochloric acid, was thymolized and then dialyzed in running water until all traces of chlorides were removed. The resultant neutral solution of paranuclein was concentrated on the water bath to a thick syrup, and while still warm was precipitated with 95 per cent alcohol. Upon standing for some time the precipitate was filtered, extracted thoroughly with alcohol and ether and dried at 105° C.[1] It gave the following analysis:

$$Ash = 13.33\%.$$

Total phosphorus $= 1.65\%$.   Phosphorus found in ash $= 1.73\%$.

Phosphorus in substance in organic combination $= 0.0\%$.

Preparation B. — Paranuclein was placed in water and sufficient sodium hydroxide added to hold the substance in solution. By repeated filtration a perfectly clear solution was obtained, and this was precipitated with acetic acid. The resulting precipitate was washed by decantation with distilled water, filtered and the substance redissolved and reprecipitated twice according to the method just described. After the final precipitation the paranuclein was treated with alcohol and ether, extracted with ether in a Soxhlet apparatus

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[1] This method of procedure agrees quite closely with that described by Chittenden, *loc. cit.*

for several days and finally dried at 105° C. Analysis gave the following results:

$$Ash = 1.68\%.$$

Total phosphorus = $\begin{cases} 2.75\%. \\ 2.67\%. \end{cases}$ Phosphorus found in ash = 0.0%.

Phosphorus in substance in organic combination = 2.75%.

DIGESTION II.

This digestion was carried out in a manner analogous to that already described with the exception that all treatment of the resultant paranuclein with alkali as well as the subsequent process of dialysis was avoided. The analysis of the product thus prepared without further purification follows:

Preparation C. —

$$Ash = 2.91\%.$$

Total phosphorus = 2.04%.   Phosphorus found in ash = 0.0%.

Phosphorus in substance in organic combination = 2.10%.

DIGESTION III.

In order to obtain further light on the influence which the method of preparation might exert upon the analysis and composition of the product, paranuclein was again prepared according to the method outlined in Digestion I. Portions of the same product were treated as follows:

Preparation D. — This preparation corresponds to preparation **A**. It served as a standard for comparison with the products to follow. Analysis gave:

$$Ash = \begin{cases} 11.80\%. \\ 11.92\%. \end{cases}$$

Total phosphorus = $\begin{cases} 2.82\%. \\ 2.90\%. \end{cases}$ Phosphorus found in ash = 2.47%.

Phosphorus in substance in organic combination = 0.44%.

It will be noticed that as in the case of preparation **A** the ash content was very high and in correspondence with the latter nearly all of the phosphorus was recovered in the ash.

Preparation E. — This preparation received treatment similar to preparation **C**, the final digestive product, however, having been simply dissolved in 1 per cent sodium carbonate solution, filtered, precipitated with hydrochloric acid, again filtered, washed and prepared for analysis.

$$Ash = \begin{cases} 1.54\%. \\ 1.67\%. \end{cases}$$

Total phosphorus = $\begin{cases} 2.40\%. \\ 2.44\%. \end{cases}$ Phosphorus found in ash = 0.10%.

Phosphorus in substance in organic combination = 2.36%.

Preparation F. — This preparation was treated like **B** with the additional feature that it was subjected to dialysis after repeated solution and reprecipitation by means of acid. It differs from preparation **E** only in the fact that it was carefully neutralized before dialysis. Analysis gave the following results:

$$\text{Ash} = \begin{cases} 1.07\% \\ 1.09\% \end{cases}$$

$$\text{Total phosphorus} = \begin{cases} 2.97\% \\ 2.77\% \end{cases} \qquad \text{Phosphorus found in ash} = 0.14\%.$$

$$\text{Phosphorus in substance in organic combination} = 2.75\%.$$

As in the case of **E**, the ash content was relatively small and practically all the phosphorus existed as organic phosphorus, i.e., disappeared on ignition.

The following summary will assist in the comparison of the analyses of the products obtained.

| Preparation. | Ash. % | Phosphorus | | | Method used in preparation. |
		In Para-nuclein. %	In Ash. %	In Ash-free substance. %	
A	13.33	1.65	1.73	0	{ Chittenden's — precipitation with alcohol.
B	1.68	2.71	0	2.75	{ Redissolved in minimal quantity of alkali and reprecipitated with acid.
C	2.91	2.04	0	2.10	No purification after digestion.
D	11.86	2.86	2.47	0.44	Chittenden's.
E	1.60	2.42	0.10	2.36	{ Similar to **C** — reprecipitated once with acid.
F	1.08	2.87	0.14	2.75	Similar to **B**; subsequent dialysis.
G	0.66	2.35	0	2.36	{ Preparation **D** dissolved in alkali and reprecipitated with acid.
H			3.03		{ Preparation **E** treated with a soluble calcium salt.

It will be observed at a glance that the retention of phosphorus in the ash of the preparation, and accordingly the *apparent* absence of organic phosphorus in the substance, is associated with those preparations only which have a high ash content (**A** and **D**). That this condition is not attributable to the dialysis of the product is evidenced by the fact that **A**, **D**, and **F** were each subjected to the process and that the ash content of the preparation is in no way related

to this part of the procedure — **F** being one of the purest products obtained, whereas **A** and **D**, dialyzed under similar conditions, showed a very high ash content. The only respect in which the preparations **A** and **D** differed materially from **F**, was in the fact that, whereas the former were precipitated from their concentrated neutralized solutions by the use of alcohol, **F** (like all the preparations showing low ash content) was first precipitated by means of acid before subsequent treatment with alcohol and ether.

It seemed reasonable to assume that the high content of ash present in preparations **A** and **D** was due to the simultaneous precipitation of relatively insoluble salts, such as calcium salts, or more probably to the fact that when the precipitation takes place in a *neutralized solution* of paranuclein, the latter is carried down in combination with an alkali earth such as calcium. Since the paranuclein behaves like an acid substance, it is reasonable to suppose that it may enter into loose combination with calcium, forming compounds analogous to those of casein with calcium and the alkalis. In this event the large amount of calcium present in the preparation would on ignition tend to hold the phosphorus originally present as organic phosphorus in the form of calcium phosphate in the ash. The discrepancy between the results of Chittenden and other observers would thus be attributable to the fact that he precipitated the paranuclein in combination with calcium or an alkali, and owing to the reaction above described, was led to assume the absence of organic phosphorus in the paranuclein.

In order to test still further the validity of this assumption, a portion of the paranuclein with high ash content (preparation **D**) was redissolved in 1 per cent sodium carbonate solution and reprecipitated with hydrochloric acid. After filtration the precipitate was washed with distilled water until the washings gave no test for chlorides.

Preparation G. — An analysis of this substance after treatment with alcohol and ether and drying at 105° C. gave the following results:

$$\text{Ash} = 0.66\%.$$

Total phosphorus $= 2.35\%$.   Phosphorus found in ash $= 0.0\%$.

Phosphorus in substance in organic combination $= 2.35\%$.

It will be seen that the preparation obtained in this way was an extremely pure product. Thus it is quite possible to transform the paranuclein compound with a high ash content, *i. e.*, paranuclein-calcium (or paranuclein-sodium), into a paranuclein containing only a very small proportion of ash and readily giving off its entire phos-

phorus on ignition. The reverse process — the formation of a calcium compound of paranuclein and the consequent cleavage of the phosphorus as inorganic phosphorus on ignition — is further demonstrated by the following experiment.

Preparation H. — A portion of preparation **E** was dissolved in calcium hydroxide. After evaporation of the fluid to a small volume, it was treated with absolute alcohol, and the precipitate thus formed removed by filtration. Analysis of this product, dried at 105° C., resulted as follows:

Phosphorus found in the ash = 3.03%.

SUMMARY.

It is shown that the paranuclein obtained by digestion of casein with pepsin-hydrochloric acid always contains a considerable content of phosphorus in organic combination. The results of previous investigators who found that the phosphorus recovered in the ash of their preparations was equivalent to the total phosphorus content of the paranuclein, are attributable to the high ash content of their products. When the latter is avoided, and thus the formation of inorganic phosphate during ignition is precluded, paranuclein invariably yields over 2 per cent of organic phosphorus.

In conclusion, it is my desire to express my indebtedness to Professor Lafayette B. Mendel for many kind suggestions.

FURTHER EXPERIMENTS ON ARTIFICIAL PARTHENO-GENESIS AND THE NATURE OF THE PROCESS OF FERTILIZATION.[1]

By JACQUES LOEB.

[From the Hull Physiological Laboratory of the University of Chicago.]

IN my previous communications on the subject of artificial parthenogenesis[2] I had confined myself to the proof of the fact that the unfertilized eggs of Arbacia, and Strongylocentrotus franciscanus and purpuratus, are capable of a development into the pluteus form if kept for from one to two hours in a mixture of equal parts of a $\frac{2}{4} n$ $MgCl_2$ solution and sea-water. The above-mentioned solution, which brings about the artificial development of the egg, differs in three directions from the constitution of the normal sea-water. First, the osmotic pressure of the solution is higher than that of the normal sea-water; second, one-half of the salts contained in normal sea-water are removed. It might be possible that the sea-water contains ions which are injurious to the development, and that the removal of these ions makes the development of the unfertilized eggs possible. Third, a considerable amount of $MgCl_2$ is brought into solution, and it might be that the Mg ions have a specific "stimulating" effect upon the development. For the determination of the nature of the process of fertilization it was necessary to find out which of the three conditions is essential for the production of artificial parthenogenesis.

2. I had already mentioned in a previous paper that the mere change in the constitution of the sea-water, if not accompanied by an increase in its osmotic pressure, can only cause the egg to go through a few segmentations, but cannot cause the parthenogenetic production of a blastula or a later stage of development. The increase in the osmotic pressure of the solution is therefore an essential condition

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[1] These experiments were carried out with the aid of the Elizabeth Thompson Science Fund.

[2] LOEB: This journal, 1899, iii, p. 135; 1900, iii, p. 434; Science, 1900, xi, p. 612.

for artificial parthenogenesis. As the season was at an end it was not possible for me to decide last autumn whether the other two above-mentioned conditions are equally essential. Through the aid of the Elizabeth Thompson Fund I was enabled to carry on experiments in coöperation with Dr. W. E. Garrey at Pacific Grove during the spring,[1] and I have since had a chance to continue this work at Woods Hole. My new results enable me to give a more definite answer to the question of the nature of the process of fertilization. I first tried to ascertain whether the $MgCl_2$ plays a specific rôle in artificial parthenogenesis, or whether its place may be taken by some other salt. I found that the latter is the case. A mixture of equal parts of a $\frac{1}{2}n$ NaCl solution and sea-water, or of equal parts of a $\frac{1}{2}n$ KCl solution and sea-water, is just as effective, if not more so than a $\frac{2}{3}n$ $MgCl_2$ solution. Unfertilized eggs of Strongylocentrotus, if left for 70 minutes in any of these solutions, developed, and some of them reached the pluteus stage. Such eggs remained alive as long as ten days. Even a mixture of equal parts of a $\frac{2}{3}n$ $CaCl_2$ solution and sea-water brought about the development of the eggs, but it was necessary to take the eggs out in about 40–50 minutes, as otherwise the solution killed them. None of the eggs treated with the $CaCl_2$ solution developed beyond the blastula stage, or lived longer than one day.

I noticed that in these experiments with a $\frac{1}{2}n$ NaCl or KCl solution only a comparatively small number of eggs reached the blastula stage, certainly many less than in my previous experiments with $MgCl_2$ on Arbacia. A further examination revealed the fact that the $MgCl_2$ solution which I had used was, through an error or a misunderstanding of the assistant who made it, weaker than a $\frac{2}{3}n$ solution. As soon as I found this out I started experiments with more diluted NaCl and KCl solution. Instead of using equal parts of a $\frac{1}{2}n$ NaCl or KCl solution and sea-water, I used the following mixtures:—

20 $\frac{2}{3}n$ NaCl — 30 distilled water + 50 sea-water,

or—

17½ $\frac{2}{3}n$ NaCl + 32½ distilled water + 50 sea-water.

In both cases more eggs reached the blastula and pluteus stage than with the original stronger mixture. In one case unfertilized eggs

[1] I wish to express my thanks to Professor Jenkins, of Stanford University, for kindly allowing me the use of the Hopkins Laboratory.

developed beautifully after having been for two hours in a solution of equal parts of 15 $2\frac{1}{2} n$ NaCl + 35 distilled water + 50 sea-water. But this was nearly the lowest limit for artificial parthenogenesis in Arbacia. As a rule 25 per cent or more of the unfertilized Arbacia eggs reached the blastula stage.

3. It was thus proved that $MgCl_2$ does not play a specific rôle in the production of artificial parthenogenesis. It remained for me to decide whether it is essential to remove one part of the normal constituents of the sea-water or whether the mere increase of the osmotic pressure suffices. I found that the increase in the osmotic pressure of the sea-water is all that is needed. In the experiments in which the maximal number of unfertilized eggs reached the blastula stage about 1 gram NaCl had been added to the sea-water. We can produce the same increase in the osmotic pressure of the sea-water by adding 10 c.c. of the $2\frac{1}{2} n$ NaCl or $2\frac{1}{2} n$ KCl solution[1] to 90 c.c. of sea-water. In this case the mixture contains practically all the constituents of normal sea-water. Yet if unfertilized eggs of Arbacia are left in such a solution for from one and one-half to two hours as many as 50 per cent of the eggs may reach the blastula stage when put back into normal sea-water. Many of these eggs die in the blastula stage and only a small number reach the gastrula or pluteus stage. The blastulæ are like those which I described in one of my former papers.[2] In the majority of cases more than one blastula develops from one egg. I have seen as many as six moving blastulæ arise from one egg. The tendency to give rise to more than one embryo is greater in the egg of Arbacia than in the egg of Strongylocentrotus. This difference is probably due to the fact that even the unfertilized egg of Strongylocentrotus often forms a fine membrane which is much thinner than the one produced through the entrance of a spermatozoön but which is sufficient to keep the blastomeres together. The addition of NaCl or KCl to sea-water favors the formation of this membrane.

4. In all the experiments mentioned thus far the increase in the osmotic pressure had been brought about by the addition of electrolytes. This might be considered as an indication that the electrically charged ions in the sea-water play an important rôle in the production of parthenogenesis. I myself was originally inclined to such

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[1] My $2\frac{1}{2} n$ NaCl solution contained 146.25 grams in a litre. The $2\frac{1}{2} n$ KCl solution contained 186.25 grams in a litre.

[2] LOEB, J. : This journal, 1900. iii, pp. 460, 461.

an assumption. I have convinced myself, however, that an increase
in the osmotic pressure of the sea-water through the addition of
cane sugar or urea can produce parthenogenesis. My stock solu-
tion of cane sugar (rock candy) was 2 *n* and contained 684.3 grams
in a litre, while the stock solution of urea was 2½ *n* and contained
150.31 grams in a litre. I found that the unfertilized eggs of Arba-
cia were able to develop after they had been for from one and one-
half to two hours in one of the following solutions : —

> I. — 100 sea-water + 25 2 *n* cane sugar.
> II. — 82½ sea-water + 17½ 2½ *n* urea.

Both the sugar solution as well as the urea solution injured the
eggs, the urea solution much more than the sugar solution. I made
an attempt to produce parthenogenesis by submitting unfertilized
eggs to a pure cane-sugar solution whose osmotic pressure was about
equal to that of the sea-water, to 90 c.c. of which 10 c.c. of a 2½ *n*
NaCl solution had been added. When the unfertilized eggs of Arba-
cia were put for about two hours into a mixture of 60 2 *n* cane
sugar + 40 distilled water or 55 2 *n* cane sugar + 45 distilled
water many of them segmented and a few developed into swimming
blastulæ, but they died within the first twenty-four hours. *This
proves conclusively that the development of the unfertilized egg is pro-
duced through an increase in the concentration of the surrounding solu-
tion. As it is immaterial whether the increase in the osmotic pressure
is brought about by electrolytes or non-conductors there can be no doubt
that the essential feature in this increase in the osmotic pressure of the
surrounding solution is a loss of water on the part of the egg.'*

5. Having reached the conclusion that the loss of water or rather
the loss of a certain amount of water causes the parthenogenetic
development of the egg, it seemed possible to take another step in
advance. In all the previous experiments the unfertilized eggs had
been submitted to a solution of higher osmotic pressure for from one
to two hours, and were then put back into normal sea-water to develop.
If the initial loss of water on the part of the egg were all that is
required for the production of artificial parthenogenesis it would be
possible to find a solution which would not only take away water
from the egg but which would also allow development to go on. I
remembered from my earlier experiments on the effects of an increase
in the concentration of sea-water upon development [1] that so slight

[1] LOEB, J.: Journal of morphology. 1892, vii, p. 253.

an increase in the concentration of sea-water as is sufficient to induce parthenogenesis allowed the development of the eggs to go on for at least twenty-four hours. *I found that if we put unfertilized eggs into a mixture of 93 sea-water and 7 $2\frac{1}{2} n$ NaCl solution many eggs develop in this solution and some of them even reach the blastula stage and swim about.* If we use a mixture of 90 sea-water and 10 $2\frac{1}{2} n$ NaCl solution the development stops earlier for the simple reason that such a solution is more injurious. These facts show clearly that the function of the artificial solution in the production of parthenogenesis is *that it has to deprive the egg of a certain amount of water*. In the majority of cases the solutions that produce such an effect are at the same time too injurious to allow the egg to develop or live long enough to reach the blastula stage. This is the reason why we have to take the eggs out of this solution and bring them back into normal sea-water, if we wish them to develop into normal larvæ.

6. A consequence of the loss of water on the part of the egg is an increase in its osmotic pressure. The osmotic pressure inside the egg is furnished chiefly or almost exclusively by electrolytes. It is thus not impossible that the ions in the egg, if their concentration is raised, bring about that change which causes the egg to develop. If we assume that the spermatozoön starts the development of the egg in the same way as in the case of artificial parthenogenesis it follows that the spermatozoön must possess more salts or a higher osmotic pressure than the eggs. As I pointed out in a former paper, this seems to be the case. But there is no reason why the spermatozoön should not bring about the same effects that we produce by reducing the amount of water in the egg in some different way. At present, however, the only light that can be thrown upon the nature of the process of fertilization must be expected from an analysis of the effects of a loss of water upon the egg.

It seems as if the liquefaction of the nuclear membrane and other constituents of the nucleus were a prerequisite for cell division. Norman showed that a certain increase in the concentration of the sea-water brings about a distribution of the chromosomes in the egg. Morgan's observations agree with this. But as all these observations were made with solutions whose osmotic pressure was considerably higher than that of the solutions used in my experiments, new observations will be required to decide this question. Hoppe-Seyler, in

one of his papers, points out that a loss of water on the part of the protoplasm brings about a diminution in the processes of oxidation. We know that lack of oxygen can bring about the liquefaction of solid constituents. I add these remarks for those who enjoy the speculative side of biology. But at the best a theory cannot give us anything more than the facts it includes, and it is therefore clearly our task to supply the lacking experimental data in this field of biology before we begin to theorize.

7. I think we should try to discover first of all whether the process of development can be started by depriving the egg of water in a few forms only, or whether this is a general condition. I have thus far tried among the sea-urchins Arbacia and Strongylocentrotus franciscanus and purpuratus. Each of these forms is capable of osmotic parthenogenesis. I am confident that the same is true for all species of sea-urchins, although the optimal increase in the osmotic pressure of the surrounding solution may vary for different forms. But I consider it of more importance that with the same methods I have been able to produce artificial parthenogenesis in a star-fish (Asterias Forbesii). By putting the unfertilized eggs of this star-fish for about two hours into a mixture of 88 c.c. of sea-water and 12 c.c. of a $2\frac{1}{2}\,n$ NaCl solution the eggs can be forced to develop and reach the blastula stage, if put back afterwards into normal sea-water. I have not yet found the optimal condition for the parthenogenetic development of Asterias, but the facts thus far obtained suffice to state that a certain increase in the osmotic pressure of the surrounding solution (and a loss of a certain amount of water on the part of the egg) causes the egg of this form to develop parthenogenetically.

I have mentioned in another place [1] the precautions and control experiments used to guard against the presence of spermatozoa. I do not consider it necessary to repeat these statements in this paper, but will mention one additional precaution for which I am indebted to the Collector of the Marine Biological Laboratory, Mr. Gray. Mr. Gray selects the females of Arbacia for my experiments, so that in all these later experiments I have not had one male in the laboratory. Not one egg developed in the control material. All the sea-water used in these experiments was heated to the temperature of 70° C.

[1] LOEB, J.: Science, 1900, xi, p. 612.

Conclusions.

The results of my experiments are as follows: —

1. Through a certain increase in the osmotic pressure of the surrounding solution the unfertilized eggs of some (probably all) Echinoderms (Arbacia, Strongylocentrotus, Asterias) can be caused to develop into normal blastulæ or even plutei.

2. This increase in osmotic pressure can be produced by electrolytes as well as by non-conductors. It is therefore probable that the parthenogenetic development is caused by the egg losing a certain amount of water.

MAMMALIAN SMOOTH MUSCLE. — THE CAT'S BLADDER.[1]

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CONTENTS.

METHODS.

THE use of the bladder as a means of studying the reactions of mammalian smooth muscle is not entirely new. Samkowy[2] has investigated the influence of temperature on the bladders of frogs, cats and rabbits, and Capparelli[3] has studied the same preparation in dogs and rabbits by means of plethysmographic tracings.

Theoretically the strongest objection to its use lies in the fact that in general direction its fibres are far from parallel. When, however, the viscus is suspended between two metal hooks and extended by a weight of twenty grams or more, its form is so elongated that this objection is almost ruled out; while the uniformity of the results obtained, the regularity in the response to a stimulus, and the fact that the whole muscle responds at once, seem to make the objection still more insignificant. As a decided advantage, the bladder may

[1] Read in abstract before the American Physiological Society, Dec., 1899: see This journal, 1900, iii, pp. xxv–xxvi.

[2] SAMKOWY: Archiv für die gesammte Physiologie, 1874, ix, pp. 399–402.

[3] CAPPARELLI: Archives italiennes de biologie, 1882, ii, pp. 291–302.

be used either *in situ*, without any disturbance in circulation, or excised, without any direct injury to the muscle substance further than that necessitated by piercing at each end with a suspending hook.

Used *in situ*, the method of preparation is as follows: The cat is anæsthetized under a bell-jar with ether, stretched on its back on an animal holder of the usual form, and tracheotomized to facilitate the continued use of the anæsthetic. The abdomen is then opened in the middle line for a distance of six or eight centimetres from the symphysis pubis. The bladder is raised in the fingers and, if full, emptied by gentle pressure.

To obtain graphic records of the contractions of the bladder an arrangement of the form shown in Fig. 1 is used. This consists of a heavy iron standard which can be moved to a position close beside the animal board. To its rod are clamped horizontal bars carrying a second vertical rod which is thus brought to stand more directly above the animal. At the lower corner of the extension is an obliquely placed rod terminating below in a sharp spike; and at some distance above is a bearing for the long recording lever. The spike, about one centimetre in length, is run through from the ventral side in the middle line of the base of the bladder; the apex is drawn upward and pierced by a small hook which is, in turn, connected by an adjustable attachment with the short arm of the lever. The lever is directed at right angles to the circumference of the smoked drum, and its point rises and falls in a straight line, not in the arc of a circle.
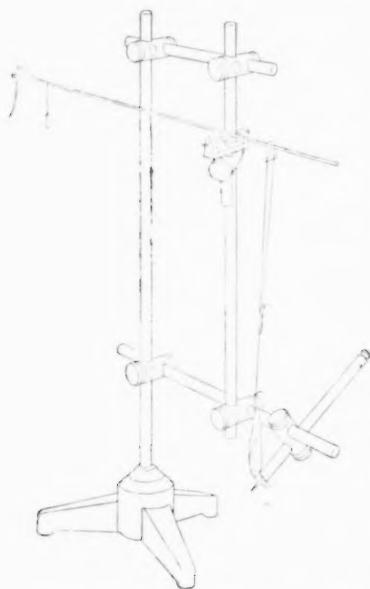
FIGURE 1 — Apparatus for suspending the bladder and recording its movements in the living anæsthetized animal.

By depressing the lower end of the spiked rod, the tissue below the point of fixation may be left so loosely relaxed that none of the

body movements, either of respiration or those of irregular origin, are communicated in the slightest degree to the recording lever. As an additional precaution, to avoid possible interference by intestinal peristalsis, it has been found best to empty the rectum by stripping it upward between finger and thumb; it should then be tied and clamped to the side of the abdominal opening. The electrical current used as a stimulus is applied through two wires, one leading to a binding post in the upper end of the oblique rod, and another, not shown in the figure, passing to the hook at the upper end of the bladder.

The advantage of this method of studying the responses of the bladder to stimulation lies chiefly in the absence of any sign of fatigue. The circulation being undisturbed it is possible to continue a series of observations for several hours without any appreciable change in the conditions. During the winter months, however, with the temperature of the room at 19° or 20° C., it is impossible to maintain the normal temperature in the exposed muscle, the resulting reactions being those of the viscus kept at 29 to 30 C. This is true even if the organ be surrounded by a casing packed with absorbent cotton kept warm by the frequent use of saline solution at body temperature.

To obtain records from the excised bladder the animal is killed with ether or curare, the abdomen is opened, and, the bladder being raised in the fingers, the urethra is cut across a few millimetres below the sphincter muscle. The urine is then expelled and the muscle transferred to a moist chamber. It is there suspended between an adjustable metal hook, insulated in the hard-rubber top of the chamber, and a second hook below, S-shaped and several centimetres in length, which in turn is attached to the long arm of the recording lever. The lever is a direct recording one exactly similar to that used with the bladder *in situ*. The electrical connections for this preparation are made by a binding-post attached to the hook in the top of the chamber, and a wire fastened to the lower, S-shaped, hook. The wall of the moist chamber is double, and the space of about two centimetres between the sides is filled with about 660 c.cm. of water. The temperature within the chamber may be kept constant, or varied at will, by heating or cooling the water as desired.

Throughout the present research both the above methods have been freely used. Where the nature of the experiment was such that even a small amount of fatigue would interfere with the result, the

bladder was left in position in the living anæsthetized animal. Otherwise the excised preparation was employed. Even with the latter the fatigue is very slight, and appears only after repeated stimulation. No differences have been found in the responses of the muscle under these different conditions. A bladder freshly exposed in the living animal reacts exactly as does one at the same temperature in the moist chamber. Nor does the nature of the result change if the animal be poisoned with curare, or if the bladder be excised until all nerve cells are dead.[1]

SPONTANEOUS MOVEMENTS.

Occasionally on opening the abdomen of a freshly anæsthetized cat the bladder will be found to be contracting and relaxing, apparently rhythmically, with a periodicity of about 45–50 seconds. This, however, is rare; in general the viscus is at rest. But spontaneous contractions occur very frequently after the muscle has been stimulated for some time.

Since Engelmann[2] in 1869 published his work on the rabbit's ureter, rhythmical spontaneous movements have been observed in other smooth muscle preparations in almost every case. Spontaneous contractions of the bladder have been described by Mosso and Pellacani,[3] Ashdown,[4] Sherrington,[5] Langley and Anderson,[6] Griffiths,[7] and others. Sherrington has shown that the spontaneous contractions of the monkey's bladder originate within its own substance. Using various preparations Engelmann,[8] Sertoli,[9] Bottazzi,[10] and Straub[11] have maintained that the spontaneous activity is purely

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[1] LANGENDORFF: Centralblatt für Physiologie, 1891, v, p. 131.

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1869, ii, pp. 243-292.

[3] MOSSO AND PELLACANI: Archives italiennes de biologie, 1882, i, pp 97-128.

[4] ASHDOWN: Journal of anatomy and physiology, 1887, xxi, pp. 299-324.

[5] SHERRINGTON: Journal of physiology, 1892, xiii, pp. 628-772.

[6] LANGLEY and ANDERSON: Journal of physiology, 1894, xvi, pp. 410-440.

[7] GRIFFITHS: Journal of anatomy and physiology, 1894-95, xxix, pp. 254-275.

[8] ENGELMANN: Archiv für die gesammte Physiologie, 1869, ii, pp. 243-292.

[9] SERTOLI: Archives italiennes de biologie, 1883, iii, pp. 78-94.

[10] BOTTAZZI: Contributi alla fisiologia del tessuto di cellule muscolari, Firenze, 1897; Archives italiennes de biologie, 1897, xxvi, p. 443; 1897, xxviii, pp. 81-90: Lo sperimentali, 1897, li, pp. 99-170; Archives italiennes de biologie, 1899, xxxi, pp. 63-68: 1899, xxxi, pp. 97-126.

[11] STRAUB: Archiv für die gesammte Physiologie, 1900, lxxix, pp. 379-399.

muscular, while Ranvier,[1] Morgen,[2] Schultz,[3] and Barbéra[4] have contended that it is of nerve cell origin. Rhythmical movements of the mammalian bladder are certainly present many hours after excision of the preparation, a fact which seems to leave no doubt as to their intrinsically muscular nature.

At times there is a regular sequence in the automatic movements of the bladder muscle, with the separate contractions of uniform size. More often they are irregular. Fig. 2 shows tracings obtained from spontaneously active preparations. The first, or upper curve (*a*) was obtained from a comparatively fresh muscle in a moist chamber at room temperature (20° C.). The time intervals are ten seconds. The second line (*b*) shows the tracing from the same muscle twenty hours after excision, the conditions remaining unchanged in the interval, and the contractions being c o n t i n u o u s. It will be noticed in both that the tracings show contractions
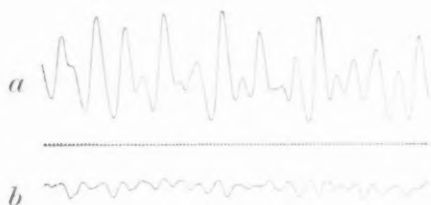
FIGURE 2.—A record of automatic bladder contractions: (*a*) from a fresh preparation and (*b*) from the same preparation 20 hours later. Time is marked in 10 second intervals. One half the original size.

which are symmetrical; the speed of contraction being approximately equal to the speed of relaxation. This form of curve has been pointed out by Sertoli[5] in records obtained from the erector penis muscle, and Ducceschi's[6] tracings from the pyloric end of the stomach have a similar form. Other observers, with non-mammalian preparations, have described the curve of spontaneous contraction as showing a relaxation phase very much longer in time than the phase of contraction. It is possible, then, that the symmetrical contraction is characteristic of mammalian smooth muscle.

[1] RANVIER: Leçons d'anatomie générale sur le système musculaire, Paris, 1880.
[2] MORGEN: Untersuchungen aus der physiologischen Institut der Universität Halle (Bernstein's), 1890, ii, pp. 139-169.
[3] SCHULTZ: Archiv für Physiologie, 1897, pp. 322-328.
[4] BARBÉRA: Zeitschrift für Biologie, 1898, xxxvi, pp. 239-258.
[5] SERTOLI: Archives italiennes de biologie, 1883, iii, pp. 78-94.
[6] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, pp. 121-189; Archives italiennes de biologie, 1897, xxvii, pp. 61-82.

Occasionally the sequence of large and small contractions gives evidence of being compounded of two or more regular rhythms, as in the cases reported by Bowditch,[1] and later by Woodworth,[2] for the frog's stomach preparation. The tracing of the first line in Fig. 3 shows very clearly the presence of two rhythms, one slightly slower than the other, the resulting record being produced by the interference of the two series of contractions working upon the lever at the same time. The second tracing shows a primary series, and a secondary series of smaller contractions at a slightly slower rate. The third, an apparently irregular curve, can be analyzed into two simple rhythms, the rates of which are indicated by the two horizontal series of dots above the recorded curve.

There is also some further evidence to support the view that this compounding of rhythms points to the occurrence of regular rhythmical contractions, with different rates in different parts of the viscus. For example, when a bladder at rest begins to show such spontaneous activity the first evidence of the disturbance is nearly always an almost perfectly uniform and rhythmical series, as though only one area were contracting. Such a tracing appears in the upper line (*a*) of Fig. 4. And again, where the bladder is kept under observation until all contractions cease, the last sign of spontaneous activity, corresponding probably to the last contracting area to die, is a faint but regular rhythmical series of contractions. The second line (*b*)

FIGURE 3.—Tracings showing compound rhythm: the first and second showing the interference of two rhythms at slightly different rates, the third showing the presence together of a slow and a fast rhythm, as indicated by the horizontal series of dots above the record. Time is marked in 10-second intervals. One half the original size.

[1] BOWDITCH: Report of the British Association for the Advancement of Science, 1897, pp. 809–810.

[2] WOODWORTH: This journal, 1899, iii, pp. 26–44.

of Fig. 4 shows such a tracing obtained from an excised preparation after thirty-one hours.

There is thus a striking analogy between the spontaneously contracting bladder and the fibrillating heart, for if the heart be observed closely during fibrillation it will very often be seen that, while the whole mass appears to be moving irregularly, yet any particular small area of the surface, watched by itself, is contracting rhythmically. The irregular spontaneous contractions of the bladder are not normal, and possibly depend on the same or similar disturbances in nutrition or in conduction as those which may give rise to fibrillation in the mammalian heart. The graphic record, also, of the
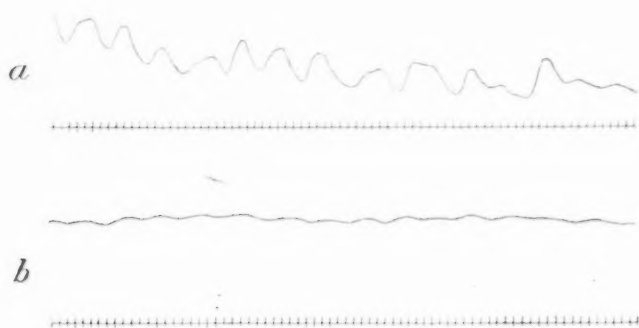
FIGURE 4. — Tracings showing (*a*) the first evidence of spontaneous activity in a newly excised bladder, and (*b*) the last, 31 hours later. Time intervals are 10 seconds. Original size.

movements of a fibrillating heart resembles the curves already shown for the bladder in spontaneous activity. Although the irregularities are much more numerous, there is occasionally an evidence of simple rhythm in the presence of an insistent beat larger than the others.

It is difficult to say what may be the purpose of automatic movements occurring normally in the living animal. It may be that such a type of activity enables the bladder to adjust its size more easily to the ever increasing amount of its contents. And it is possible that, stimulated to greater force by distention, the spontaneous movements serve to expel into the urethra the first few drops of urine which there arouse the desire to urinate, and start the reflex nervous mechanism for the emptying of the bladder.

THE SINGLE CONTRACTION.

The bladder responds to mechanical stimulation, to stimulation by means of single induction shocks, to the making and the breaking of the constant current, and to the action of the constant current during the flow. It is also influenced by changes in temperature, though it is not clear whether this last result consists in an actual contraction, or is merely a change in tonic condition.

The graphic record of a single contraction, in response to a single induction current, presents the characters already described by earlier workers for other smooth muscle preparations. Fig. 5 shows the form of the contraction obtained from an excised bladder at body temperature on stimulating with a single break induction current of moderate strength. A latent period of about 0.25 second duration is followed by a relatively
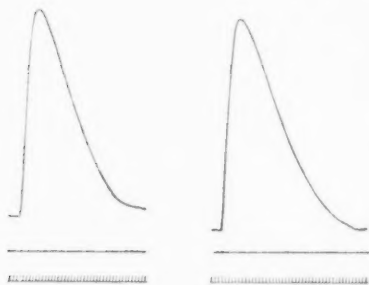
FIGURE 5. — Contractions of the bladder muscle at body temperature in response to single induction currents. The lever is magnifying by $5\frac{1}{2}$. Time is indicated in seconds. Original size.

rapid rise of the lever, lasting typically five or six seconds. This is succeeded by a more gradual relaxation usually complete in about thirty-five seconds. The end of the relaxation, in particular, is very slow. Although there are variations in the size of the contraction the time of the whole process is regularly about forty seconds. To compare with this we have Sertoli's[1] determination for the erector penis muscle of the dog, 90–120 seconds, and Lewandowsky's[2] finding for the nictitating membrane in cats, — a contraction lasting from five to fifteen seconds, followed by a relaxation from three to eight times as long, variations occurring with the size of the contraction.[3]
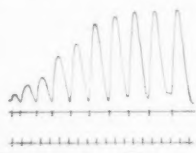
FIGURE 6. — The contraction increasing with the stimulus. Coil at 13.5, 13, 12.5, 12, 11.5, 11, 10.5, 10, 9.5, and 9 cm. Time in 10-second intervals. Original size.

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[1] SERTOLI: Archives italiennes de biologie, 1883, iii, pp. 78–94.

[2] LEWANDOWSKY: Archiv für Physiologie, 1899, pp. 352–359.

[3] For other smooth muscle preparations see CAPPARELLI: Archives italiennes

With an increase in the strength of the stimulus the height of the contraction increases up to a maximum. The curve in Fig. 6 shows the response to a series of single break induction currents with the secondary coil at 13.5, 13, 12.5, 12, 11.5, 11, 10.5, 10, 9.5, and 9 cm. from the primary. At 13.5 cm. the contraction is very small, but increases progressively until the coil is at 10 cm. Beyond that point there is no increase, the contraction being maximal.

SUMMATION OF CONTRACTIONS AND TETANUS.

If the excised bladder at body temperature be stimulated by means of two equal induction shocks of moderate strength, and separated by known intervals, it is found that when the interval is greater than about eight seconds, the result is shown in the form of two separate contractions. If the interval be less than eight seconds, however, summation takes place, and, as in striped muscle, the effect of the second stimulus is added to that of the first. The fusion of the two curves in the record of such a pair of contractions becomes more and more complete the shorter the interval. Fig. 7 shows a series of curves obtained in this way, with intervals varied from one to eight seconds, preceded by a single
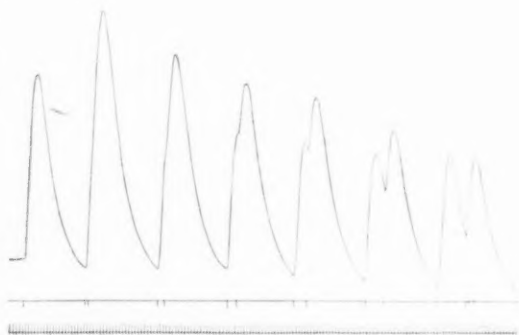
FIGURE 7. — A record showing a single contraction and the effect of two equal stimuli with intervals of 1, 2, 3, 4, 6, and 8 seconds. Bladder excised, at body temperature. Time in seconds. Original size.

de biologie, 1882, ii, pp. 291–302; MORGEN: Untersuchungen aus der physiologischen Institut der Universität Halle (Bernstein's), 1890, ii, pp. 139–169; SCHULTZ: Archiv für Physiologie, 1897, pp. 307–321; BARBÉRA: Zeitschrift für Biologie, 1898, xxxvi, pp. 239–258; WINKLER: Archiv für die gesammte Physiologie, 1898, lxxi, pp. 357–398; BOTTAZZI and GRÜNBAUM: Journal of physiology, 1899, xxiv, pp. 51–71; POMPILIAN: Comptes-rendus de la société de biologie, 1899, i, p. 489; and STRAUB: Archiv für die gesammte Physiologie, 1900, lxxix, pp. 379–391.
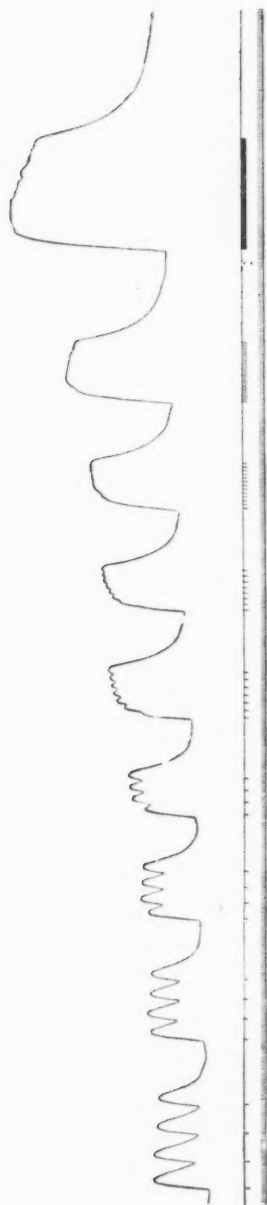
FIGURE 8. — The development of tetanus. Induction currents of moderate strength applied at intervals of 10, 8, 6, 5, 4, 3, 2, 1, and ⅓ seconds, respectively. Time in seconds. Seven-ninths the original size.

contraction. It is found that at one second there is no apparent difference between the result of two stimuli and that of a single stimulus, except that the contraction is higher. With an interval of two seconds, however, a slight irregularity in the rising arm of the contraction curve can be detected, indicating the incomplete fusion of the two waves. As the interval is increased the separation is more evident, until at eight seconds summation fails. It is also to be observed that where there is no summation with the longest interval there is still an overlapping of the curves. To produce summation the second stimulus must fall either during the first contraction or *early* in the relaxation phase. The tracing shows also, as the result of temporary fatigue following such frequent stimulation of the viscus, a slight falling off in the height of the initial contraction.

If a series of equal stimuli be used, summation takes place in the same way.[1] Fig. 8 shows the record of

[1] For the development of tetanus in smooth muscle see ENGELMANN: Archiv für die gesammte Physiologie, 1870, iii, pp. 247-326; CAPPARELLI: Archives italiennes de biologie, 1882, ii, pp. 291-302; SERTOLI: *Ibid.*, 1883, iii, pp. 78-94, who has found a single induction shock inefficient as a stimulus for the erector penis muscle; PAWLOW: Archiv für die gesammte Physiologie, 1885, xxxvii, pp. 6-31; BOTTAZZI: Archives italiennes de biologie, 1897, xxviii, pp. 81-90; BARBÉRA: Zeitschrift für Biologie, 1898, xxxvi, pp. 239-258; WINKLER: Archiv für die

an experiment in which the bladder at body temperature was stimulated with break induction currents of moderate strength, with varied time intervals between the currents. On shortening the interval, summation appears, and becomes more and more pronounced until, with an interval of one second between the stimuli, complete fusion occurs. Beyond this point, as shown in the last curve of the tracing, the result of stimulation increases as the interval between the stimuli decreases. The tetanus curve thus obtained appears to be exactly similar to that of striped muscle, except in time relations. In all cases after such prolonged stimulation the muscle is fatigued and relaxes very slowly.

There appears to be no period in the contraction of the bladder muscle, occurring either spontaneously or in response to an excitation, during which a stimulus is without effect. Ducceschi[1] has observed a refractory period in the spontaneous rhythmical contractions of the pyloric end of the stomach. He records complete failure of response if the stimulus be applied during the phase of rising energy, and a variable result if stimulated during the first half of the fall.

THE EFFECT OF THE CONSTANT CURRENT.

Throughout the experiments upon the effect of stimulation by means of the constant current a battery of two small storage cells was used. It supplied a current of from four to five volts, and about three milliampères passed through the bladder suspended in the moist chamber.

Both the making and the breaking of the constant current act as stimuli and produce contractions, though the contraction in response to the make is generally of much greater extent than that following the break. This is in agreement with almost all previous experimenters, though the result of the breaking of the current has been variously described.

As to the disputed question whether the constant current, during its flow, acts as a stimulus, there can be no doubt as far as the bladder muscle of the cat is concerned. The effect of the flow of the

gesammte Physiologie, 1898, lxxi, pp. 357-398; WOODWORTH: This journal, 1899, iii, pp. 26-44; and STRAUB: Archiv für die gesammte Physiologie, 1900, lxxix, pp. 379-399.

[1] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, pp. 121-189; Archives italiennes de biologie, 1897, xxvii, pp. 61-82.

current is often sufficient to obscure partially the break contraction. Fig. 9 gives a demonstration of this. To obtain the tracing, which is one of a large number, the current is made, allowed to flow for several seconds, and then broken. The result is a strong make contraction, succeeded by a period of very slow, imperfect relaxation, the true relaxation occurring only after the break contraction. That this tonic elevation, following the make contraction, is due to the flow of the current is proved by the second curve of the figure. Here the same current was made in the same way, but shut off gradually with a rheonome, avoiding both the influence of the flow and the break contraction.

If, again, with a bladder muscle at rest, the constant current be turned both on and off with the rheonome, there occurs a slight rise in tonus during the flow, though the electrical change is too gradual to produce either the make or the break contraction. Sertoli[1] has described a tonic relaxation, with cessation of the spontaneous contractions of the erector penis preparation, during the flow of a weak constant current; Ducceschi[2] has observed the same inhibition of spontaneous con-
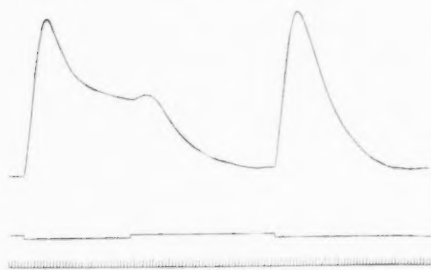
FIGURE 9. — A record showing the effect: first, of the make, flow, and break of the constant current; second, of the make alone. Time in seconds. Original size.

tractions in the pyloric end of the stomach; Ranvier[3] has noted a slight inhibition of the movements of the frog's stomach preparation, and Winkler[4] has occasionally observed a relaxation. This I have been unable to find in the bladder preparation. With the tonic contraction during the flow there is often a decrease in the size of the spontaneous movements, but not enough to be explained otherwise than by the temporary shortening of the whole muscle.[5]

[1] SERTOLI: Archives italiennes de biologie, 1883, iii, pp. 78–94.
[2] DUCCESCHI: Archivio per le scienze mediche, 1897, xxi, pp. 121–189.
[3] RANVIER: Leçons d'anatomie générale sur le système musculaire, Paris, 1880
[4] WINKLER: Archiv für die gesammte Physiologie, 1898, lxxi, pp. 357–398.
[5] Professor Theodore Hough, of the Massachusetts Institute of Technology, has told me, and kindly allows me to repeat, that he has observed this inhibiting

I have been unable to find any direct evidence of an antagonism between the make and the break excitations with the constant current. Winkler [1] has described a relaxation as the result of breaking the current, but this result is probably only the failure of an evident break contraction, and a relaxation as the result of the mere stopping of the flow. Woodworth [2] has observed relaxation on making the current in the frog's stomach preparation. Observations bearing on this question have been made by Engelmann [3] and Capparelli,[4] using mammalian preparations, by Biedermann,[5] and by Woodworth,[6] with the frog's stomach, who have found that the extent of the response to stimulation with the constant current varies, within limits, with the duration of the flow. Woodworth (*loc. cit.*) states that between the make and the break " there must be a perceptible interval in order to get any response. And as the interval is increased, the response increases " (p. 41). Woodworth advances this as a proof of the antagonism between the make and the break, and has demonstrated that it is not the result of varying the duration of the flow, by reversing the process, passing the current through the muscle, and stimulating by means of a break followed by a make. In this case the result varies directly with the duration of the *pause* in the flow.

This finding is fully confirmed by the reaction of the bladder muscle preparation, and is more clearly demonstrated than in the published tracings of those who have established the essential facts. In Fig. 10 two tracings from the bladder muscle are reproduced. The upper tracing shows the effect of making and breaking the constant current with a series of intervals of $\frac{1}{25}$ (estimated), $\frac{1}{2}$, 1, 5, and 25 seconds. It will be noticed that with $\frac{1}{25}$ second there is no response, and that the contractions increase in size up to the 5-second interval. The tracing at 5 seconds is higher than the one following, because, with that interval, there is summation of the make and break contractions. In

effect of the constant current upon the automatic contractions of the frog's stomach preparation. Prof. Hough's tracings show, during the passage of the current, a great decrease in the amplitude of the automatic movements.

[1] WINKLER : Archiv für die gesammte Physiologie, 1898, lxxi, pp. 357–398.
[2] WOODWORTH : This journal, 1899, iii, pp. 26–44.
[3] ENGELMANN : Archiv für die gesammte Physiologie, 1870, iii, pp. 247–326.
[4] CAPPARELLI : Archives italiennes de biologie, 1882, ii, pp. 291–302.
[5] BIEDERMANN : Elektrophysiologie, Jena, 1895.
[6] WOODWORTH : This journal, 1899, iii, pp. 26–44.

the lower tracing of the figure the current is flowing, sufficient time
having elapsed for the disappearance of the effect of the original
stimulus. Then the current is cut off for intervals of $\frac{1}{20}$, $\frac{1}{5}$, 1, 5, and
10 seconds, as indicated in the record. The first produces no effect,
and then, as the *pause* in the flow is lengthened, the response in-
creases, the summated contraction at 5 seconds being again higher
than the succeeding one. To prevent interference by fatigue, these
experiments were made on fresh preparations, with the circulation
intact. For the same reason the series was arranged with the weak-
est stimuli first. With the excised bladder at body temperature the
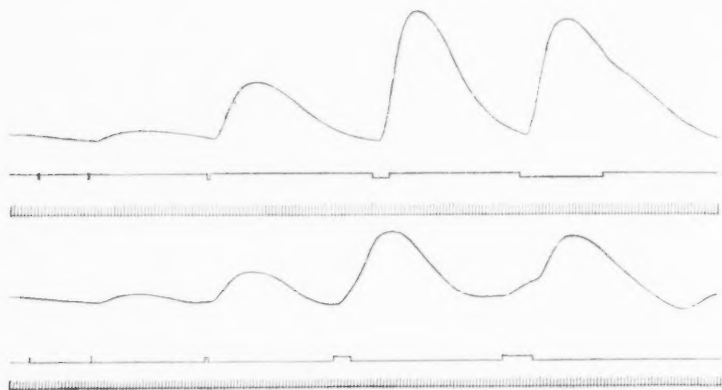
FIGURE 10. — In the upper line, the effect of the make followed by the break, with
intervals of $\frac{1}{20}$, $\frac{1}{5}$, 1, 5, and 25 seconds: below, the effect of the break followed
by the make, intervals of $\frac{1}{20}$, $\frac{1}{5}$, 1, 5, and 10 seconds, the current flowing mean-
while. Time in seconds. Original size.

result is exactly the same, except that it is found almost impossible
to stimulate for so short a time that no response will result, this dif-
ference being due to the greater irritability of the preparation at
38° C. as compared with the bladder *in situ* at 30° C.

We have demonstrated the separate effects of both the making and
the breaking excitations, and have shown that the effects may be
summated. And it has been impossible to find any trace of relaxa-
tion as the direct result of stimulation by means of either the make
or the break under any conditions. It is difficult, therefore, to accept
an explanation for these results which postulates an antagonism
between the two stimuli in their effect on smooth muscle.

THE INFLUENCE OF TEMPERATURE.

1. **On the tone of the bladder muscle.** — If the bladder muscle, enclosed in the moist chamber of a water jacket, be cooled, it is found to shorten considerably with the lowering of the temperature. At about 10° C. the shortening is complete. If the temperature be now slowly raised, relaxation appears almost immediately, but proceeds very slowly at first, and becomes more marked only when the temperature rises to about 15–19° C. The loss of tone then proceeds regularly to a temperature of about 40° C., that is, slightly above the body temperature. Above 40° C. shortening once more appears, and proceeds, slowly at first, then more rapidly, until the temperature of the moist chamber is raised to from 53° to 57° C. At this point the muscle apparently loses its irritability and dies. There follows then, whether the muscle be kept at that temperature or heated further, a distinct loss of tone often of considerable extent.
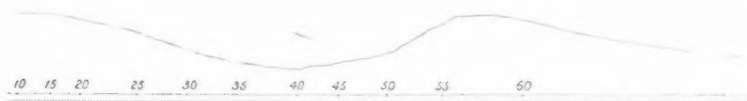
FIGURE 11. — The curve of tonic change, with temperature rising, as indicated, from 10° to 60° C. Time intervals 10 seconds. One-third the original size.

In this stage the muscle is comparatively relaxed, and very soft. It is only when the temperature is raised to 69° C. that the first shortening of heat rigor is obtained.

Considering the whole process, then, from the standpoint of the condition of the muscle at the body temperature, it may be said that cold produces an increase of tonus, while heat gives first a slight relaxation, then a marked increase in tone. In relation to the condition of the muscle at a temperature slightly higher, however, the muscle may be said to contract for both heat and cold.

In the experiments from which these results have been obtained, every care was taken to make the changes in temperature slowly, so the bladder might have time to be fully warmed. And control experiments were made with small isolated strips of the muscle.

Fig. 11 shows the typical form of the curve of change of tone with a resting bladder. The temperature, beginning at 10° C., was slowly raised as indicated in the tracing. The record shows very clearly a maximum lengthening in the neighborhood of 40° C., and demon-

strates the loss of tone following the
loss of irritability, in this case at 56° C.[1]
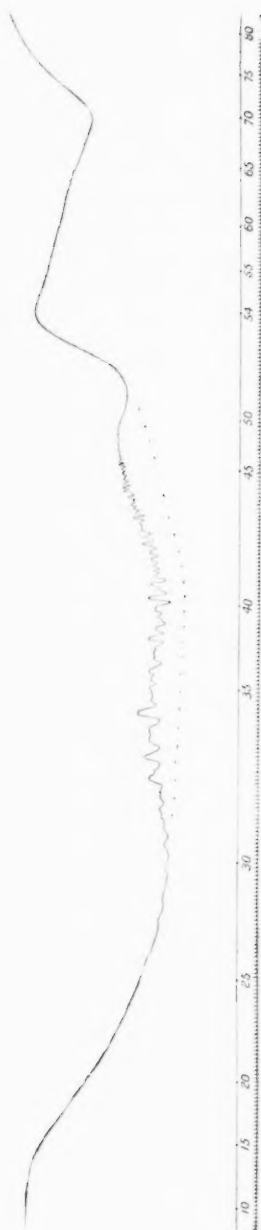
The influence of changes in tempera-
ture upon the spontaneous contractions[2]
of the bladder muscle is also very
marked. Entirely absent at 10° C.,
they appear early in the course of the

[1] For the literature bearing upon this subject
see SCHMULEWITSCH: Journal de l'anatomie et
de la physiologie, 1868, pp. 27-47: Medicinische
Jahrbücher, Wien, 1868, xv, pp. 3-36; Comptes-
rendus de l'académie des sciences, 1869, lxviii,
pp. 936-938; GRÜNHAGEN and SAMKOWY:
Archiv für die gesammte Physiologie, 1875. x,
pp. 165-171; SERTOLI: Archives italiennes de
biologie, 1883, iii, pp. 78-94; GRÜNHAGEN:
Lehrbuch der Physiologie, 1886; BOTTAZZI:
Archives italiennes de biologie, 1899, xxxi, pp.
97-126, and WOODWORTH: This journal, 1899,
iii, pp. 26-44, who regard the change in tempera-
ture as a stimulus: SCHUR: Zeitschrift für
rationelle Medizin, 1868, xxxi, 373-403: SAM-
KOWY: Archiv für die gesammte Physiologie,
1874, ix, pp. 399-402; MORGEN: Untersuch-
ungen aus der physiologischen Institut der
Universität Halle (Bernstein's), 1890, ii, pp.
139-169: SCHULTZ: Verhandlungen der physi-
ologischen Gesellschaft zu Berlin, 1895-96;
Archiv für Physiologie, 1896, pp. 543-544;
BRODIE and RICHARDSON: Journal of physi-
ology, 1897, xxi, pp. 353-372: Philosophical
transactions of the Royal Society, 1899, vol.
191, pp. 127-146: BOTTAZZI and GRÜNBAUM:
Journal of physiology, 1899, xxiv, pp. 51-71,
and VERNON: Journal of physiology, 1899, xxiv,
pp. 239-287, who has noted the relaxation with
the loss of irritability.

[2] See ENGELMANN: Archiv für die gesammte
Physiologie, 1869, ii, pp 243-292: CAPPARELLI:
Archives italiennes de biologie, 1882, ii, pp. 291-
302; SCHULTZ: Archiv für Physiologie, 1897,
pp. 322-328; BOTTAZZI: Archives italiennes de
biologie, 1899, xxxi, pp. 97-126; BOTTAZZI and
GRÜNBAUM: Journal of physiology, 1899, xxiv,
pp. 51-71; WOODWORTH: This journal, 1899,
iii, pp. 26-44.

FIGURE 12. — Curve of change of tone showing the apparent irregularity due to the presence of spontaneous contractions. One-half the original size.

first relaxation on warming, although at low temperatures they are feeble and very slow. As the temperature rises toward that of the body, the contractions become more frequent and are of greater extent. Beyond a maximum slightly below body temperature, further warming increases the rapidity and frequency of the movements, but diminishes their amplitude, until they disappear entirely with the loss of irritability at a temperature in the neighborhood of 55° C.

Where automatic contractions are present in the preparation, irregularities in the course of the tonus curve are introduced. This is the case in the record shown in Fig. 12. With the appearance of spontaneous activity the curve, instead of falling regularly to 40° C., as in the dotted line, shows a secondary rise. This is due to the fact that in such a condition some part of the bladder is always in contrac-
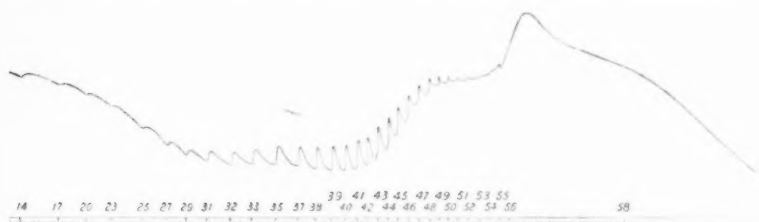
FIGURE 13. — Curve of change of tone with rising temperature, showing the variation produced by frequent stimulation. One-third the original size.

tion, the result being an *apparent* rise in tone which increases with the amplitude of the movements. And again, on raising the temperature beyond 40° C., when the contractions so diminish in amplitude that they no longer of themselves sustain the lever, there is an apparent secondary loss of tone. This form of curve is characteristic of such preparations, and in other respects is perfectly in accordance with the result shown in Fig. 11. Fig. 12 shows also the loss of tone following the rise to 54° C., and the true contraction of heat rigor, appearing at 69° C.

If the muscle be stimulated during the course of an experiment on the influence of temperature on the tone of the muscle, another characteristic variation from the true result is obtained. In Fig. 13 the progress of the change in tone is interfered with in the same way as in the case illustrated in Fig. 12, but for a different reason. The muscle was stimulated every few degrees, as indicated in the record,

with a single induction current, for the purpose of testing the irrita-
bility of the preparation. The variation is due to the after effect, or
contraction remainder, following the application of the stimuli. As
the temperature rises the effect becomes greater with the increase of
irritability, and is particularly marked above 40° C. When the irri-
tability begins to disappear the lever sinks back to its true course,
and once more records the real condition of the muscle with respect
to tone. The tracing is also of interest because it shows the final
loss of tone which accompanies the permanent loss of irritability at
about 57° C.

These irregularities are constant, and are dwelt upon because it is
believed that in overlooking them and their cause very important
errors are made in studying the influence of temperature.

2. **On the form of the contraction.** — The changes in the form of the
contraction curve of smooth muscle, resulting from changes in tem-
perature have already been described by various authors.[1] With the
cat's bladder the facts are in accordance with the results previously
obtained.   The first sign of irritability in response to a single
induction current appears at a temperature of about 10° C.   At 15°
C. the contraction is slight and, in relation to its extent, very slow.
The contraction at 20° C. is more active, the latent period is shorter,
and the maximum height is earlier reached.   As the temperature
rises the latent period shortens progressively,[2] and the height of the
contraction increases to an optimum temperature of about 40° C.
Above the optimum point the height decreases until irritability entirely
disappears at, or about, 55° C. With the rise in temperature there is a
steady increase in the steepness of the rise of the curve; and this
depends only in part on the increased height of the contraction of cer-
tain temperatures, for there is also a progressive shortening of the time
of the contraction phase.   There is also, as the temperature rises, an
increase in the steepness of the descent of the lever, particularly in

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[1] For mammalian smooth muscle, GRÜNHAGEN and SAMKOWY: Archiv für die
gesammte Physiologie, 1875, x. pp. 165–171 ; CAPPARELLI: Archives italiennes
de biologie, 1882, ii, pp. 291–302, and SERTOLI: Archives italiennes de biologie,
1883, iii, pp. 78–94: for non-mammalian, MORGEN: Untersuchungen aus der
physiologischen Institut der Universität Halle (Bernstein's), 1890, ii, pp. 139–169,
SCHULTZ: Archiv für Physiologie, 1897, pp. 1–28, and WINKLER: Archiv für die
gesammte Physiologie, 1898, lxxi, pp. 357–398.

[2] The latent period at 10° C. is 3.5 seconds; at 15° C., 2.5; at 20° C., 1.6; at
25° C., 0.75 ; at 30° C., 0.4; at 35° C., 0.3: at 38° C., 0.25 ; at 40° C., 0.2, from the
averages of a number of determinations.

the early part of the relaxation. And, finally, the time of the relaxation process shortens considerably.

Fig. 14 is a reproduction of a series of actual curves obtained at various temperatures from 15° C. to above 50 C., under conditions otherwise uniform.

The variations in the height of the contraction must be considered in connection with the general variations in tone of the bladder
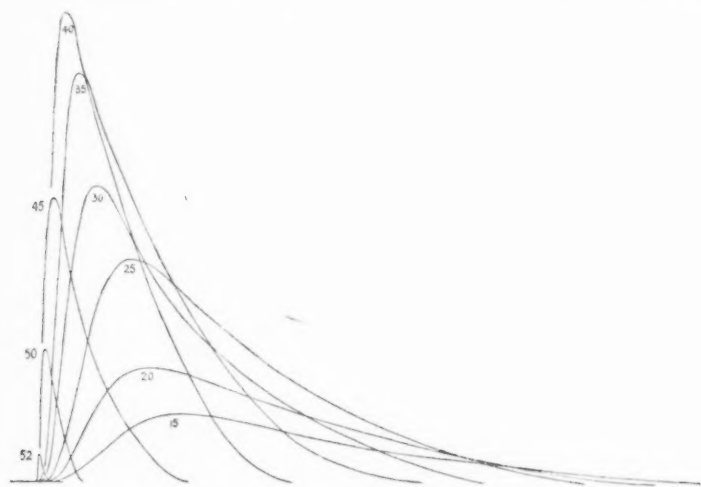
FIGURE 14. — Temperature and the form of the curve. The numbers represent degrees C. Time in seconds.

muscle, already referred to. When the muscle is in a state of tonic contraction, the contractions resulting from stimulation are necessarily decreased in height, though the total shortening of the muscle may actually be greater than the height of the maximum contraction at 40° C., where the tonic relaxation is also greatest. The height of the contraction at any temperature cannot, therefore, be considered as an absolute index of the irritability of the muscle at that temperature.

THE ELASTICITY OF THE MUSCLE AND THE INFLUENCE OF THE LOAD ON THE HEIGHT OF THE CONTRACTION.

Under the influence of an increase in weight the bladder elongates very considerably. The changes in length take place more slowly

than with striped muscle under the same conditions, but in other particulars the results are exactly similar. Fig. 15 demonstrates the elasticity of the bladder muscle. Beginning from a position of rest with a weight of 20 grams (including the lever), the first drop in the curve shows the extension following the addition of 20 grams to the weight. Each succeeding fall shows the effect of a further increment of 20 grams, the extension being progressively less in each case. When the total weight amounted to 120 grams, all the weights were removed with the exception of the original 20 grams, and the tracing shows in the final vertical line the recovery of the normal length. The total lengthening under the greatest weight amounted to 15 mm., fully 40 per cent of the original length of the viscus.
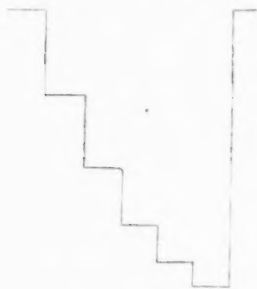
FIGURE 15. — Record showing the relative extension of the muscle under additional weights of 20, 40, 60, 80, and 100 grams. Drum turned by hand. One-half the original size.

Fig. 16 is a reproduction of a record indicating the influence of the load on the height of the contraction. The first vertical line shows the height to which the lever rises with the usual load, 20 grams; the second, the response to a single induction shock with a load of 40 grams. Each succeeding line represents the relative height for an additional weight of 20 grams until 140 grams is reached. As with striated muscle, the contraction decreases in height in a roughly geometrical ratio.

FIGURE 16. — The influence of load; the relative heights of the contractions, with loads of 20, 40, 60, 80, 100, 120 and 140 grams. Drum turned by hand.

Within the limits of the experiment, the actual output of energy, in the form of work done, is greater the greater the weight.

The Duration of Irritability ; Fatigue.

Sertoli,[1] keeping his preparations for the greater part of the time at a low temperature, has found that the erector penis muscle may remain irritable for five, six, or even seven days. Various other observers[2] have noted the extreme slowness with which smooth muscle dies under ordinary conditions. With the bladder muscle of the cat the duration of irritability at room temperatures is often as much as twenty-four to forty-eight hours, while one preparation, kept in an ice-box at 5–8° C., responded to the faradic current at the end of four days.

The bladder *in situ*, with the circulation intact, does not, in the course of any ordinary series of experiments, show any evidence of fatigue for very long periods. It does, however, show temporary
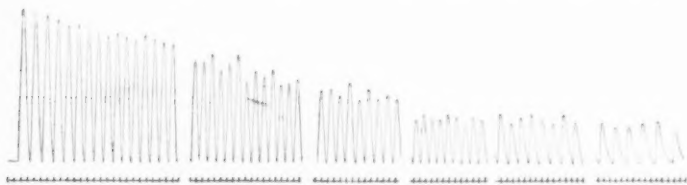
FIGURE 17. — Fatigue curve. Contractions numbered 2-17, 29-41, 55-63, 92-100, 121-128, and 149-154. Time intervals 10 seconds. Four-fifths the original size.

fatigue if strong stimuli be repeated rapidly, as in producing tetanus. Even then recovery takes place within a short interval, the time varying with the extent of the stimulation, and the experiments may be proceeded with as before. It is impossible to completely reduce the contractility of a fresh muscle even under the most powerful stimulation.

With the excised preparation the facts are different. With ordinary use for two, or even three or four hours' experimentation, the muscle lasts extremely well, giving at the end of that time almost

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[1] Sertoli: Archives italiennes de biologie, 1883, iii, pp. 78-94.

[2] Capparelli: Archives italiennes de biologie, 1882, ii, pp. 291-302; Morgen: Untersuchungen aus der physiologischen Institut der Universität Halle (Bernstein's), 1890, ii, pp. 139-169: Meirowsky: Archiv für die gesammte Physiologie, 1899, lxxviii, pp. 64-86: Bottazzi: Archives italiennes de biologie, 1899, xxxi, pp. 97-126; Straub: Archiv für die gesammte Physiologie, 1900, lxxix, pp. 379-399.

as good reactions as at the beginning. But if the stimuli be repeated too frequently, without allowing any sufficient pause for recovery, the extent of the contraction will diminish, and there will be a gradual lengthening of the time of each of the phases of the contraction. This change in the response is not always very apparent within the limits of a small series of contractions, but appears if, for example, the muscle be stimulated by means of induction currents, each current falling just at the end of the relaxation following the previous contraction. Fig. 17 shows portions of a fatigue curve obtained under such conditions. At the end of each relaxation a new stimulus was applied, until the bladder was almost completely exhausted. The figure shows contractions numbered 2–17, 29–41, 55–63, 92–100, 121–128, and 149–154 of the series. In fatigue not only is the height of the contraction diminished, but the curve is lengthened materially. The lengthening is present in the latent period, in the period of contraction, and in the period of relaxation during which the lever returns to the base line. During a pause in such a series of stimuli the muscle recovers considerably, though if the series be a long one, particularly if the bladder has been stimulated to tetanus, the recovery never gives a complete return of the original force of contraction.

FIGURE 18 — Recovery with the reversal of the constant current. Ascending current for the first, third, and fifth groups of contractions, descending for the second and fourth. Time intervals 10 seconds. Original size.

Morgen [1] has noted that, with a frog's stomach preparation fatigued by the repeated application of the constant current, a recovery of the original efficiency may be obtained by reversing the current. This same peculiar result is given by the bladder, as shown in Fig. 18. In this experiment the muscle was stimulated in every case by means of a constant current lasting two seconds. For the first six contractions the current was passed from base to apex. Fatigue being evident, the current was then

[1] MORGEN: Untersuchungen aus der physiologischen Institut der Universität Halle (Bernstein's), 1890, ii, pp. 139–169.

reversed, made to pass from apex to base, allowing no pause for recovery. The next contraction shows a great increase in force as compared with the last of the previous series. Fatigue again sets in, and recovery is once more shown on changing the direction of the current. This is repeated several times, each time with the same result.

It might be concluded from such a tracing that the explanation lies in a possible polar effect of stimulation with the constant current. Engelmann [1] has observed that, with the rabbit's ureter, the make current produces a contraction beginning at the cathode, the break at the anode. If, then, the effect of the make current being the stronger, stimulation produced fatigue only, or chiefly, in the cathodal region, reversal of the current would subject a new and comparatively fresh region to the influence of the stimulus. During the interval while this second area was being fatigued, the first would recover, and so on.

That this explanation does not hold good, however, is shown by clamping the bladder midway between the two electrodes, and recording the contractions of the anodal and cathodal regions separately. In this case, both regions respond in the same way. Both respond equally to the make, both to the break. And on stimulating to partial fatigue, both show recovery with the reversal of the current. Whatever the cause may be, it is in some way a property of the muscle in its entire length. The phenomenon is therefore still unexplained.

Conclusions.

The bladder, used either *in situ* or excised, supplies an excellent preparation for the study of the properties of mammalian smooth muscle.

Automatic movements are generally present, and show at times both simple and compound rhythms. A compound rhythm is produced when two or more areas of the muscle contract rhythmically at different rates.

The bladder responds to single induction currents, the make, break, and flow of the constant current, to mechanical stimuli and to changes in temperature. The response increases to a maximum with the strength of the stimulus.

[1] ENGELMANN: Archiv für die gesammte Physiologie, 1870, iii, pp. 247-326.

The single contraction, appearing at about 10° C. and disappearing at about 55° C., shows a progressive shortening of all its phases with the rise in temperature, and a maximum height at 40° C.

Single contractions may be summated regularly, and, with an interval slightly less than two seconds, are fused to complete tetanus. There is no refractory period.

Unless the making and breaking of the constant current are separated by an interval of more than about $\frac{1}{26}$ second no result is obtained. With longer intervals the contraction increases to a maximum both with the flow and with the pause in the flow of the current.

The make and break excitations show no antagonism and their contractions may be summated.

If the muscle be fatigued by stimulation with the constant current, reversal of the current gives a recovery of the original force in both anodal and cathodal regions.

With rising temperature the bladder shows a relaxation in tone from 10° C. to 40° C. From 40° C. to about 55° C. there is a tonic shortening, maximal with the loss of irritability at from 53° C. to 57° C. There appears, then, a relaxation, without rigor, persisting until the true contraction of heat rigor appears at 69° C.

With the rise in temperature spontaneous contractions appear, are maximal in amplitude below normal body temperature, and increase progressively in rate until they disappear with the death of the muscle at about 55° C.

The muscle shows perfect elasticity, and, with the increase in weight shows, within limits, an increased output of energy in the form of work done.

The bladder *in situ* lasts indefinitely, and shows at most only slight temporary fatigue. The excised bladder may be used for several hours, fatigues with repeated stimulation, but shows partial recovery. Spontaneous contractions persist for from twenty-four to forty-eight hours at room temperature; and the cooled muscle may maintain its irritability for as much as four days.
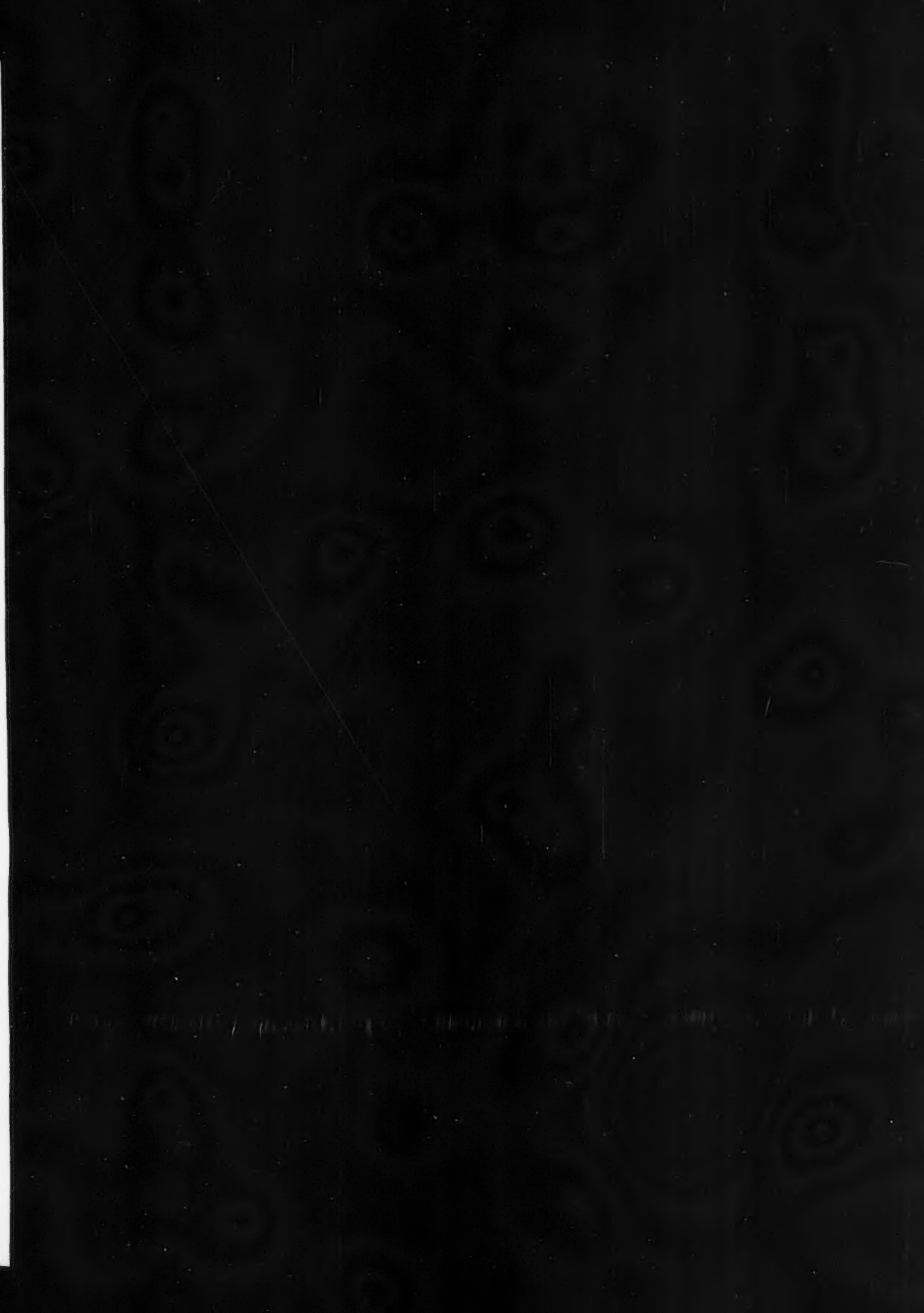
ON THE PHYSIOLOGICAL ACTION OF THE POISONOUS SECRETION OF THE GILA MONSTER (*HELODERMA SUSPECTUM*).

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[*From the Physiological Laboratory of the Johns Hopkins Medical School.*]

CONTENTS.

THE experiments here recorded were undertaken in continuation of an investigation begun by one of us in 1896 and brought to a sudden close by the death of the only Gila Monster at that time available as a source of venom. To this earlier paper,[1] and to one by Santesson,[2] the reader is referred for the more general facts concerning the poison of these remarkable lizards.

These more recent experiments were performed upon dogs, cats, and frogs. With the exception of the first three experiments, all of the dogs were anæsthetized with ether after an injection of morphine. The cats received ether alone. Respiration was recorded with a tambour tied to the abdominal wall just below the sternum, and arterial pressure, with a mercury manometer writing on an endless roll kymographion. The Hürthle manometer was used to determine the strength of the heart-beat.

Three fine active Helodermas, obtained from Arizona in the summer of 1899, furnished the venom used. The quantity obtainable from each monster varied much from time to time, ranging from about one to seven minims. It was obtained sometimes by causing the monsters to bite filter paper, sometimes by allowing them to bite

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[1] VAN DENBURGH: Transactions American Philosophical Society, XIX, 1898, p. 199.

[2] SANTESSON: Nordiskt medicinskt Arkiv, Key-Festband, 1897, pp. 1-48.

directly upon rubber and letting the poison drop from this into a small dish. The largest Heloderma was about nineteen inches in length.

We wish especially to express our sense of indebtedness to Dr. William H. Howell, in whose laboratory this investigation was carried on, for many kindnesses and for suggestions to which this paper owes much of whatever value it may possess.

GENERAL SYMPTOMS.

When Heloderma poison is injected into unanæsthetized normal dogs the general symptoms are very similar to those observed in pigeons, and are, in the main, readily explained by the facts brought out in the more detailed considerations which follow. The three experiments recorded here show how grave and complicated these symptoms may be, and with what appalling promptness they may follow the entrance of the poison into the blood current.

Experiment I, Jan. 16, 1900. — Mongrel fox-terrier.

10.33.15 A. M.   All the poison (six or seven drops) obtained from one monster was injected in left groin, subcutaneously.

10.38.   Respirations 26 per minute.

10.39.   Pulse 89.

10.47.   Respirations 28.

10.52.   Respirations 25.   Dog scarcely uses left hind leg in walking.

10.48.   Micturition, defecation, and dribbling from the mouth.

11.03.   Defecation.

11.12.   Continual slight shivering.

11.14.   Defecation.   Respirations 27.

11.16.   Still shivering and dribbling saliva.

11.43.   Respirations 30.

11.57.   Sat down, apparently with some tenderness in left hind leg, and immediately afterward lay down.

12.07.   Respirations 27.

1.05.   Respirations 34.

1.43.   Respirations 37.

1.44.   Vomited a little pale yellowish liquid.

4.13.   Respirations 32.   Has vomited considerable grayish watery liquid since 1.45.

5.22.   Respirations 32.

5.25.   Vomited again.

About 10.45 the dog began to show signs of drowsiness and stood perfectly still with his head hanging low, his nose almost touching the floor. He seemed to be temporarily roused by noises, whistling, etc., but after showing brief interest, by raising his head and occasionally by wagging his tail, soon reassumed the characteristic posture with pupils somewhat enlarged. This apparent drowsiness increased slightly during the next two or three hours, and the dog became more and more inclined to lie down quickly after being disturbed. At no time, however, was control of his muscles lost even to the slightest degree. Later in the afternoon the dog seemed brighter, and, although he lay down quickly after being disturbed, he did not seem to be greatly affected by the poison, but wagged his tail readily when spoken to. He appeared very thirsty and drank copiously when given water at 3.30.

Jan. 17. — The dog was found dead and cold at 9 A. M.

Experiment II, Feb. 13, 1900. — Small mongrel pug. The right external jugular vein was exposed under cocaine, and at 11.26.03 A. M. fifteen minims of normal salt solution containing five drops of fresh poison were injected, probably into the connective tissue behind the vein.

11.51.   Micturition.

11.58.   Micturition and defecation.

12.01.   Micturition.

12.04.   Defecation.

12.05.   Micturition and defecation.

12.07 and 12.09.   Micturition.

12.13.   Defecation.

12.16.   Micturition and dropping of saliva.

12.17.   Defecation.

12.20 and 12.27.   Micturition.

12.29.   There being no apparent effects of the first dose aside from general restlessness and frequent attempts at micturition and defecation, six minims of undiluted poison from another Heloderma were injected intravenously. Before the dog could again be placed on the floor he had slight convulsive contractions of the leg muscles. When placed on the floor, he immediately crawled under a box and fell on his side unconscious; respiration was paralyzed within one-half minute after injection.

12.33.   Pulse rate 64 per minute.

12.34–36.   Convulsive movements and twitchings of the muscles of the neck and face, and, upon touching the sides of the body, convulsive contractions of the muscles of the thorax and abdomen were observed.

12.37.   Three deep respiratory gasps.   Pulse 134.

12.39.   Pulse rate 103.

12.40.   Two respirations.

12.42–12.43.30.   Nineteen practically normal respiratory movements.

12.43.30–12.44.45.   Five deep gasps. These were soon followed by eight deep inspiratory gasps, which were the last sign of respiratory activity.

12.47.45.   The heart stopped beating.

Experiment III. Feb. 20, 1900. — Small mongrel dog. Weight 12½ lbs. The right external jugular vein was exposed under cocaine, and five minims fresh, undiluted poison were injected intravenously at 10.40.55 A. M. The dog was immediately placed upon the floor, but was unable to stand, and lay on his side. Although evidently unconscious, he began almost at once to howl in a mechanical sort of way, with each expiration, and continued to do so until respiration temporarily stopped at 10.42.05.

10.41.30.   Micturition and shortly afterward defecation.

10.44.30.   Respiration began again quietly and easily. The pupils were greatly dilated.

10.46.   Corneal reflex nearly normal. Abdominal muscles tense and thorax apparently contracted.

10.48.   Muscles of the limbs, trunk, and neck contracted locally when touched, as if shrinking from contact.

10.49.30.   Pulse 114.

10.50.30.   Pulse 97.

10.51.   There has been no respiration for more than a minute and a half. The legs are stiffened.

10.51.25.   Three slight respiratory movements.

10.51.40.   Began to howl again, the respirations being somewhat convulsive, the snout drawn toward the chest and the mouth opened and tongue protruded with each expiration.

10.51.47.   Twenty-one respirations per minute.

10.53.30.   Corneal reflex very slight.

10.54.40.   Pulse rate 133.

10.57.   The howling has ceased gradually, as have also the respiratory movements, which now are confined to a mere drawing back of the upper and lower lips. During this minute (10.57) there were eight of these lip movements, — each slighter than the preceding, — the last signs of respiratory activity.

10.57.30.   Heart-beats very weak. Corneal reflex gone.

10.58.20.   Heart-beat can no longer be felt.

11.   Thorax opened. Heart still beating, but without co-ordination of its auricles and ventricles; the right ventricle beats twenty-eight times, while the corresponding auricle beats but sixteen. The auricular beats are, however, much the stronger.

11.02.15.   Ventricle shows only fibrillar contractions while the auricle still beats strongly.

11.04.15.   Diaphragm responds to mechanical stimulation of phrenic nerves.

THE EFFECTS UPON RESPIRATION.

Santesson [1] and Van Denburgh [2] both have pointed out that, with moderate doses, the immediate cause of death is failure of respiration. They have suggested that the sharply contrasting conclusion reached by Mitchell and Reichert [3] may be explained by the quantity of poison employed, since it has been stated that certain snake venoms kill by paralysis of respiration when given in small doses, but by cardiac failure when injected in larger amounts. In the present series of experiments, however, failure of respiration always occurred before the heart was profoundly affected, even when enormous doses were injected intravenously.

Santesson, working with mice, noticed dyspnœa preceding the failure of respiration. Van Denburgh, experimenting upon pigeons, observed often a very great increase in the number and force of the respiratory movements, followed by a more or less gradual paralysis of respiration; — phenomena which he thought might be due to stimulation of the respiratory centre by changes in the oxygen-carrying power of the blood. This preliminary quickening, followed by gradual paralysis, has in the present investigation been found to occur quite constantly in dogs (see experiments below). It may also be observed in frogs (Exp. VIII) but in these animals respiration frequently stops almost immediately after injection.

The stimulation of respiration comes on so quickly, is so transient and recurs so regularly after repeated doses of venom (see Exp. VII, etc.) that it can hardly be due to changes in the blood. That it is a result of the sudden fall in blood-pressure seems equally improbable, since, on repeated injection, the quickening of respiration sometimes appears when the fall in pressure has amounted to not more than 5 mm. Hg (Exp. VII). Mitchell and Reichert [4] have shown that a similar effect produced by the venom of the Crotalidæ is due to peripheral vagus stimulation, but this explanation will not hold for Heloderma poison, since the stimulation is quite as apparent when the vagi

[1] SANTESSON: *Loc. cit.*

[2] VAN DENBURGH: Transactions American Philosophical Society, 1898.

[3] MITCHELL and REICHERT: Medical news, 1883, xlii, pp. 209-212. "This interesting and virulent heart poison contrasts strongly with the venoms of serpents, since they . . . cause death chiefly through failure of the respiration."

[4] MITCHELL and REICHERT: Smithsonian contributions to knowledge, 1890, xxvi, p. 122.

have been cut (Exp. IV, etc.). Peripheral stimulation of other nerves has not been definitely excluded as a cause, but it seems more than probable that both the increased activity and the gradual paralysis are to be explained by the direct action of the poison on the respiratory centre.

When very large doses have been given, inspiratory spasms sometimes occur, during which a single inspiration may last seventy or eighty seconds. Apparently, such spasms are due to tetanization of the respiratory muscles, but they seem never to last long enough to result in death (Exp. III, etc.).

The diaphragm always responds after death to mechanical and electrical stimulation of the phrenic nerves.

The vagi retain their power of inhibiting the activity of the respiratory centre.

Experiment IV, Oct. 27, 1899. — Small mongrel dog. Vagi cut.

Time. Min. Sec.	Respirations per 10 secs.	Height of curve in mm.	Remarks.
Normal	3	3	Injected five minims of fresh undiluted poison in right femoral vein. Blood-pressure 93 mm. Pulse 27 per ten seconds.
10	3.8	4	
20	6.2	3	Blood-pressure 63 mm.
30	19.5	2	
40	24.5	1.5	Blood-pressure 56 mm.
50	19.5	2	
1	15.5	3	Blood-pressure 58 mm.
1 10	12	3.5	
1 20	10	3	
1 30	9.8	3	
1 40	8.4	2	
1 50	8.	3	
2	6.6	4	Blood-pressure 64 mm.
2 10	4.2	5	
2 20	4	1 & 5.5	
2 30	3	1 & 5.5	
2 40	2	6	
2 50	1	5.5	
3	1.9	5.5	Blood-pressure 50 mm.
5	1	3	Blood-pressure 40 mm.
5 30	1.5	2	
6	2	1.5	Blood-pressure 34 mm.

Experiment IV (continued) —

Time. Min. Sec.	Respirations per 10 secs.	Height of curve in mm.	Remarks.
8 20	2	*	Blood-pressure 31 mm. Pulse 31 per 10 seconds.
10	1	*	Blood-pressure 27 mm.
10 30	1	*	Blood-pressure 22 mm.
11	0		Blood-pressure 22 mm.

* Too small to measure.

Experiment V, Oct. 31, 1899. — Small mongrel dog. Vagi cut.

Time. Min. Sec.	Respirations per 10 secs.	Height of curve in mm.	Remarks.
Normal	2	15.5	Injected one minim fresh undiluted poison in right femoral vein. Blood-pressure 115 mm. Pulse 29.5 per ten seconds.
10	2	15.5	
20	2	15.5	
30	2	8.5	Blood-pressure 87 mm.
40	2.3	12–17	
50	4	17.5–12.5	
1	5.7	11.5–1	Blood-pressure 72 mm.
1 10	6	4.5–.5	
1 20	4	3–.5	
1 30	2	1–.5	
1 40	2	.5	Blood-pressure 67 mm.
1 50	1		
2	0		
6 20	1.5	1.5	
6 40	1	1	Blood-pressure 32 mm.
9 10	1	.5	
9 50	1	+	Blood-pressure 27.5. Pulse 28.5
12 20	0		
12 30	0		Blood-pressure 12. Pulse 14 (?)
15	0		Blood-pressure 7. Pulse 0.

Experiment VI, Jan. 25, 1900. — Large mongrel dog. Vagi not cut. Respiration rapid and irregular.

Time. Min. Sec.	Respirations per 10 secs.	Height of curve in mm.	Remarks.
Normal	15	10.5–1	
"	9	11–1	
"	1	11	

Experiment VI (continued) —

Time. Min. Sec.	Respirations per 10 secs.	Height of curve in mm.	Remarks.
Normal	9.5	10.5–1	Injected into femoral vein half of the fresh poison taken from one monster and diluted with five minims of salt solution. Blood-pressure 144 mm.
”	7.5	7–.8	
10	6	2	
20	15	8–1	Blood-pressure 72 mm.
30	32	5–1	
40	26	2–.5	
50	24.5	3.5–1	
1	17	4.5–1	Blood-pressure 73 mm.
1 10	14.5	3–.5	
1 20	9	2	
1 30	12	1	
1 40	11	3–.7	
1 50	12	.5	
2	3	3–.5	Blood-pressure 64 mm.
2 10	8	.5	
2 20	2	.5	Pen stopped writing.
7	1	2.5	Blood-pressure 98 mm.
7 10	2	2–.5	
7 20	3	1	
7 30	2	2–.7	
8	2	5–1	Blood-pressure 58 mm.
8 20	2	5	Repeated injection as at first.
8 40	1	4.5	
8 50	0		
9	0		Blood-pressure 50 mm.
9 10	1	4.5	
9 20	1	4	
9 30	0		
9 40	0		
9 50	2	5–3.5	
10	1	2	Blood-pressure 44 mm.
11 10	2	2.5	
12 10	2	1	Pulse 26.7
12 50	1	.7	Pulse 20.
13 15			Respiration stopped.
13 30			Pulse 8.
14			Heart stopped beating.

Experiment VII. Dec. 12, 1899. — Dog. Vagi not cut.

Time. Min. Sec.		Respirations per 10 secs.	Height of curve in mm.	Remarks.
Normal		3.4	3	Injected intravenously 0.0008 gram dried poison in 6 minims salt solution. Blood-pressure 106 mm.
	10	3	3	
	20	4	3	
	30	5.5	6	Blood-pressure 62 mm.
	40	5	4	
	50	4.2	3	
1		4.5	3.7	Blood-pressure 64 mm.
1	30	4.2	2	
2		4	1.5	
2	30	4.2	2	
3		4.8	1	
3	30	5	1.5	Blood-pressure 90 mm.
4		3	1.2	
4	20	3.5	1.2	
5	20	3.4	2	Blood-pressure 102 mm.
6	20	3	3	
7	20	3	3	
10	20	3	2	
11		3.5	3	Injected intravenously 0.0013 gram dried poison in 8 minims salt solution. Blood-pressure 104 mm.
11	30			
11	40	3.5	3	
11	50	4.5	10-2	Blood-pressure 92 mm.
12		6.5	7-3.5	Blood-pressure 86 mm.
12	30	3.5	2.5	
13		3	1.5	
14		3	2.5	
15		3.3	2.5	
26		3	6-3	
31	30	4	2	
37	50	4	1	
39		2.5	2	Injected intravenously 0.008 gram dried poison in 4 minims salt solution. Blood-pressure 109 mm.
39	50			
40		3	1.5	
40	10	3	1	
40	20	4	14-.5	Blood-pressure 96 mm.
40	30	1.5	2	
40	40	3	1	
40	50	3	1	
41	30	6	2.5	

Experiment VII (continued) —

Time.		Respira-tions per 10 secs.	Height of curve in mm.	Remarks.
Min.	Sec.			
42		4	1.5	
43		4	2–.5	
44		2.5	1	
57	40	3	1.5	
64		3	2	
70		3.5	2	
75		4	2	
96		5	.5	
97				Cut right vagus.
97	30	10.5	7–.5	
98	20	5	1.5–.5	
98	50	4	1.5–.5	
115		6	2	{ Injected intravenously 10 minims of a
115	10			{ strong solution of dried poison.
115	20	5	1	Blood-pressure 80 mm.
115	30	7	8–2.5	Blood-pressure 75 mm.
115	40	15	9	Blood-pressure 62 mm.
115	50	11	11–9	
116		10	11–5	
116	10	8	7	
117	30	5	6–2	
117	40	5	8	Left vagus cut.
118		3	14	
119		8	7	{ Injected 15 minims strong solution of dried
120	30	10	8–6	{ poison.
120	40	11	8–6	
120	50	7	8–5	
121		12	7–5	
121	10	10	6	
121	20	8	7	Experiment stopped.

Experiment VIII, Nov. 22, 1899. — Frog — moderately large *Rana pipiens.* Seven minims weak solution of dried poison injected into dorsal lymph sac.

Min.	Sec.	Remarks.
1	43	Respiration very rapid.
9		Repeated injection.
34	50	Respiration again moderate.
90	8	Injected twenty minims solution of fresh poison.
92	23	Respiration again forced.
105	38	Repeated last injection.
108	53	No respiration.

The Effects upon the Heart-Beat.

The usual effects of Heloderma poison upon the heart are similar to its effects upon respiration, but appear more slowly. At, or a little before, the time when the blood-pressure has reached its lowest point in the primary fall the pulse rate usually is quickened (see experiments below, under arterial pressure). This quickening may be very marked (Exp. XI) or may be entirely absent (Exp. XII). The Hürthle manometer shows that the heart-beats also become stronger at about this time (Exp. XIII and XIV). These changes following the fall in arterial pressure are perhaps compensatory, but they occur when the vagi and cervical cord have been cut (Exp. XIV, etc.) and, therefore, cannot be central in origin. Following this stage, the pulsations become gradually weaker and then slower, until the ventricles, and later the auricles cease beating (see Exp. III and IX). After death the right side of the heart usually is distended with blood while the left is nearly empty.

In our experiments respiration always stopped before the heart, but artificial respiration does not prevent the gradual cardiac failure.

The vagi do not lose their power to inhibit the activity of the heart when stimulated artificially (Exp. X).

Experiment IX. Nov. 1, 1899. — Dog about size of fox-terrier. Heart exposed by removing the sternum, and opening the pericardium. Artificial respiration throughout experiment.

At 2.21.25 P. M. twelve minims of fresh undiluted poison were injected into the femoral vein. No change was observed in the action of the heart, either in rhythm or amplitude, during the time required for a fall in arterial pressure from 92 mm. Hg (normal) to 36 mm. The heart-beats then gradually became weaker. At 2.30 the heart was still beating slowly but rhythmically, while at 2.34.10 its contractions were uncoördinated. The auricles were still beating regularly at 2.36, but their contractions were followed only by fibrillar contractions in the ventricles. Soon after this the auricles also stopped.

Experiment X. Oct. 26, 1899. — Dog. Vagi cut. Cannula in carotid. Injection into right femoral vein.

Time. Min. Sec.	Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks
Normal.	94	21.5	5	(Injected five minims moderately (strong solution of dried poison.
5	77			

Experiment X (continued)—

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
	10	52	21.7	5	
	20	47	22.3	5	
	30	49	24.5	5	
	40	49	26	4	
	50	49	27	4	
1		48	26	4	
1	10	46	26.5	4	
1	20	44	26	4	
9	50	34	24	1	
10	40	34	23	1	
11	30	33	23	1	
11	40	17	5	1	{ Stimulated peripheral end of the
11	50	41	21	1	{ vagus seven seconds.
12		37	25	1	
12	10	36	26	1	
23		37	25	1	
71		17	23.4	1	

Experiment XI, Mar. 2, 1900. — Dog. Vagi not cut. Cannula in carotid. Injection into femoral vein.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal.		123	14.3	3	{ Injected moderate dose of fresh
	10	112	28.5	2	{ poison.
	20	88	35.5	2.5	
	30	84	32.5	2.5	
	40	78	31.5	3	
	50	72	32	3.5	
1		71	32.8	3	
1	10	71	34	2	
1	20	73	34.3	1.5	
1	30	75	34.3	1	
1	40	75	34.7	.7	
1	50	75	34.3	.7	
2		76	35	.7	
2	30	76	36.5	.5	Experiment stopped.

Experiment XII, Mar. 23, 1900. — Cat. Vagi cut. Cervical cord cut at occipito-atlantal membrane. Cannula in left femoral artery. Injection into right femoral vein.

Time. Min. Sec.	Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal.	14	22	1	Injected fresh poison solution
10	14	22	.8	
20	14	22	.8	
30	14	22	.8	
40	14	22	.8	
50	13	22	·7	
1	13	22	·7	
1 20	12	20·5	·5	
2	12	19·7	·3	

Experiment XIII, Feb. 6, 1900. — Mongrel bull-terrier. Vagi cut. Cannula in left femoral artery. Hürthle sound in left ventricle. Injection into right femoral vein. Artificial respiration after cutting cervical cord.

Time. Min. Sec.	Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Hürthle beats in mm.	Remarks.
Normal.	91	25·7	4	30	Cut cord.
2 20	25	22	3	6	
2 40	24	21·5	3	6	Injected 20 minims
3	22	21·3	3	6	of salt solution con-
Normal.	19	21·3	3	6	taining about six
10	18	21	3	6	drops of fresh
20	20	20·8	3	5·5	poison.
30	24	20	3	6·5	
40	23	20	3	6	
50	23	19·2	3	6	
1	23	18·5	3·5	7	
1 10	24	18·3	4	9·5	
1 20	10	14·5	5·5	10	
1 30	6	7	14	10	
1 40	10	7·2	8	8	
1 50	15	12·7	5	5·5	
2	12	13·7	5	5	
2 10	13	13	5	5	
2 20	17	12·5	4	5	
2 30	16	12·7	3·5	3·5	
2 40	18	12·7	2·5	2·5	
2 50	20	12·7	2		
3	14	13·4	2		
4	12	13	1	2·5	
4 10	8	13	1	1·5	
4 20	8			1	
4 30	7	11	·5	·5	
4 40					Animal dead.

Experiment XIV, Jan. 30, 1900. — Large mongrel setter. Vagi intact. Cannula in left femoral artery. Hürthle sound in left ventricle. Injection into right femoral vein. Artificial respiration after cutting cervical cord.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Hürthle beats in mm.	Remarks.
Normal		98	21.8	4.5		Cut cord.
						Injected 10 minims of solution
Normal		20	11.8	7	16.5	of six drops of venom in 15
	10	20	12	7	15.5	minims normal
	20	20	12	6	16	salt solution.
	30	20	12.7	5	16.5	
	40	20	12	5	17.5	
	50	24	13	5	19.5	Injected the other
1		29	12.8	4.5	18.5	five minims of the poison solu-
1	10	29	12.8	4	17	tion.
1	20	30	13.3	4	16	
1	30	30	14	3	15.5	
1	40	31	14.5	2	13.5	
1	50	31	15.5	2	13	
2		32	16.6	1.5	12.5	
2	30	31	19.3	1	11	
3		34	32	.5	9.5	
3	30	34	32.5	.5	8.5	
3	50	34	32.7	.5	7	
4	30	32	31	.5	5	
5		30	23	.7	5	
5	30	29	23.7	.7	4.5	
6		28	23.4	.7	3	
6	30	26	24	.5		
7		24	24	.5		
8		23	22	.5		
9		19	20.7	.2		
9	30	18	16			
10			0			Animal dead.

THE EFFECTS UPON ARTERIAL PRESSURE.

Almost the first effect of intravenous injection of Heloderma poison is a great and rapid fall in arterial pressure. This fall often begins within five seconds of the time when injection was begun and is so

rapid as to amount frequently to forty or fifty millimetres of mercury within twenty seconds. Following this primary fall, there usually is a moderate increase in arterial pressure, but when this occurs it is only transient, being succeeded by a gradual fall almost to zero.

The question, of course, arises whether these changes in pressure are due to variations in the peripheral resistance or to cardiac effects of the poison. In this connection experiments (Nos. XIII and XIV) with the Hürthle manometer are of interest, since they show that the strength of the heart-beat remains practically unaltered throughout the time of the first fall in arterial pressure, but that the second and more gradual fall in pressure corresponds to a gradual weakening of the heart. Moreover, when the cervical cord has been cut no immediate fall in pressure follows injection of the poison. One seems justified, therefore, in believing that the primary and secondary falls in arterial pressure are, at least to a great extent, of different origin, the former being due to vaso-dilatation, the latter to gradual cardiac failure.

The temporary increase in pressure, following the first fall, occurs nearly synchronously with an increase in the pulse rate (Exp. XV, XIX, etc.) to which probably it is chiefly due, although the Hürthle manometer shows that the heart beats a little more strongly at this time (Exp. XIII and XIV). When the dose has been small, it is probable that there sometimes is a partial recovery of vascular tone (Exp. XVI), but the temporary rise in pressure may occur after section of the cervical cord.

The evidence as to whether the vasodilatation is due to paralysis of the vaso-constrictor centre or to a peripheral action of the poison is not clear. When the cervical cord has been cut, the only fall in arterial pressure caused by injection of the poison is that parallel to the gradual failure of the heart (Exp. XIII, etc.). This fact would seem to show that the primary fall is of central origin, but, on the other hand, might be explained on the ground that any further fall was prevented by a complete dilatation of the vessels upon cutting the cord. When the pressure has been raised by electrical stimulation of the cut end of the cervical cord, injection of the poison causes a fall in pressure similar to the primary fall in normal animals (Exp. XIX, XX, XXI). This is evidence that the effect is not purely central, but the fact that injection of adrenal extract after the primary fall caused a marked rise in pressure shows that the muscle-coats of the arterioles cannot have been seriously injured (Exp. XVII). A

possible explanation is that the conductivity of the vaso-motor paths is seriously impaired.[1]

No preliminary stimulation of the vaso-constrictors, corresponding to the quickening of respiration and pulse, has been observed.

Experiment XV. Nov. 1, 1899. — Dog. Vagi cut. Heart exposed. Artificial respiration. Cannula in carotid. Injection in femoral vein.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal		90	31	1	Injected twelve minims fresh undiluted poison.
	5	86	30.5	1	
	10	61	30	1.5	
	15	56			
	30	56	32	1.5	
	40	60	32.5	1.5	
	50	59	32 5	1.5	
1		58	32.5	1.5	
1	10	55	32.5	1	
1	20	53	31.5	1	
1	40	48	33.5	.5	
2		47	33	.5	
2	20	46	33.7	.5	
2	30	45	34.5	.5	
2	40	40	33.5	.5	
3	20	36	32.5	.5	
3	30	35	32.5	.5	Experiment stopped.

Experiment XVI, Dec. 12, 1899. — Mongrel dog about size of fox-terrier. Vagi not cut.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal		106	15	6	Intravenous injection of .8 mgm. of dried poison in six minims salt solution.
	10	102	17.7	4	
	20	76	21	4	
	30	62			
	40	64	22	5	
	50	64	21	5	
1	30	68	21.3	5	

[1] Experiments XVIII, XIX, XX, XXI. show that the power of conduction is not always completely destroyed.

Experiment XVI (continued) —

Time. Min. Sec.	Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
2	72	22	4	
3	84	22.7	4	
4	93	23	3	
5 25	102	21.5	3	
6 50	102	20.8	3	
10 50	102	18	3	Injected 1.3 mgm.
11 30	104	18	3	of dried poison
11 40	104	19	3	in 8 minims nor-
11 50	92	20.3	3	mal salt solution.
12 30	84	20.3	3	
14	80	18.6	3	
15	82	19	3	
26	88	18	2.5	
37 50	101	18.8	2.5	Injected 8 mgm. of
39 50	109	20.5	1	dried poison in
40 10	109	21	1	4 minims salt so-
40 20	96	21	1.5	lution.
40	73	20.6	1.5	
41	71	20.3	1.5	
41 30	65	20.5	.5	
43	67	21.5	.5	
44	59	21	1	
46	53	20	.7	
64	57	23.3	.5	
75	66	24	.3	
96 50	75	27		Right vagus cut.
97 50	66	29	.5	
115 10	80	29.7	.5	Injected 10 minims
115 30	75	31	.5	of strong. solution
115 50	53	29	2	of dried poison.
116 10	44	28.4	1	
117 40	46			Left vagus cut.
118	40			
118 30	54	33	.2	Injected 15 minims
120 30	44	31	.2	of same solution as
120 40	43	30.5	.2	last injection.
120 50	40	31	.2	
121 20	40	30	.2	Stopped experiment.

Experiment XVII. Nov. 3, 1899. — Mongrel Irish terrier.   Vagi cut.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks
Normal		106	26.7	3.5	Injected 4 minims solution of fresh poison in femoral vein.
	10	106	26.9	3.5	
	20	77	27.4	3.5	
	30	64	27	3.5	
	40	60	27	3.5	
1	10	63	26.4	3.5	
3		74	27.2	2.7	Injected 15 minims of adrenal extract.
3	10	86	27.8	2.5	
3	20	104	28	2.5	
3	30	94	28	2.5	This was followed by several injections of poison and adrenal extract.
4		84	27.8	2.5	
58	20	51	28.4	1	Injected 10 minims of strong solution of dried poison.
58	30	50	28.2	1	
60		42	26	.5	
60	10	41	27	.5	Injected 25 minims of adrenal extract.
60	30	43	28	.5	
61		58	28.5	.5	
61	10	57			
64	30	40	28	.25	

Experiment XVIII, Nov. 2, 1899. — Mongrel fox-terrier. Vagi cut. Cannula in left femoral artery.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal		99	29	2.5	Injected .03 minim of fresh poison in five minims of salt solution into right femoral vein.
	5	99			
	10	86	29	2.5	
	20	57	30	2.5	
	40	41	30	2.5	Stimulation of the floor of the fourth ventricle with a strong alternating current 12 secs.
3		61	34.5	2	
3	10	62	35	2	
3	20	92	34.8	2	
3	25	102			

Experiment XVIII (continued) —

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
3	30	81	34.8	2	
3	40	67	35	2	{ Repeated stimulation
10	10	63	34.5	2	{ as before.
10	20	102	36.5	2	
10	25	124			
10	30	102	36.5	2	
10	40	75	37.5	2	
10	50	70	37	2	Experiment stopped.

Experiment XIX. Mar. 20, 1900. — Cat. Vagi and cervical cord cut. Cannula in femoral artery. Injection in femoral vein. Artificial respiration.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal.		127	38.5	2	Cut cord.
Normal.		53	35	1.5	{ Began stimulation of cervical cord
	10	60	34.5	1.5	{ with alternating current.
	20	66	35	1.5	
	30	74	35.5	1.5	
	50	82	38	1.5	
1	10	96	38	1.5	
1	20	95	40	1	Injected poison from one monster.
1	30	70	39	1	
1	40	60	38	1	
1	50	56	38	1	
2	10	57	37.6	.7	Stimulation off.
2	30	53	37	.5	{ Repeated stimulation, same
3	10	40	38	.5	{ strength as before.
3	20	40	38	.5	
3	50	41	40	.5	Stronger stimulation.
4	20	49	39.5	.3	
4	50	42	40	.3	
5	50	25	36	.3	
6	50	22	31	.25	
7	50	20	27	.2	Stimulation off.
8	20	19	29	.2	
10	20	17			
11	20	17			Animal dead.

Experiment XX. Mar. 27, 1900. — Cat. Vagi and cervical cord cut, artificial respiration. Cannula in left femoral artery. Injection into right femoral vein. Stimulation of cut end of cervical cord with alternating current.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal.		80	30.4	2	Cut cord.
"		37	28.5	1	Stimulation on.
		50	30.5	1	
		62	30.5	1.5	Injected fresh poison.
	10	54	29.8	1	
	20	46	30	1	
	30	46	30.4	1	
	40	43	30.5	.7	
1		40	30.5	7	
2		34	30.5	5	
4		26	29	3	Stimulation off.
5	10	20	25.5		Same stimulation on.
5	30	20	27		
6		21	28.8		Stronger stimulation.
6	30	24	28.3		
7	25	20	29.5		Stimulation off.
9		16			
12		14			Animal dead.

Experiment XXI, Mar. 28, 1900. — Cat. Vagi and cervical cord cut. Artificial respiration. Cannula in left femoral artery. Injection in right femoral vein. Stimulation of cord as in Experiments XIX. XX.

Time. Min. Sec.		Pressure. mm. Hg.	Pulsations per 10 secs.	Amplitude in mm.	Remarks.
Normal.		78	36.2	1.5	Cut cord.
"		56	32	1.5	Stimulation on.
		104	35	2	(Injected moderately strong solu-
	10	94	35.5	1.7	(tion of dried poison.
	20	73	34	1.5	
	35	54	32	1	
	45	52	33.8	.7	
	55	47	34.3	.7	
1	15	40	34.4	.5	
1	40	35	35	.5	
2	30	31	34.5	.5	Stimulation off.
3		35	35.7	.5	
14	30	28	31.3	.7	Stronger stimulation.
15		77	36.8	.8	
15	30	60	38	.5	
15	38				Stimulation off.
15	40	42			
27					Animal dead.

THE EFFECTS UPON THE BLOOD.

Globulicidal. — Heloderma poison, like many snake venoms, when mixed with blood causes the red corpuscles to swell so that they lose their biconcave form and appear spherical and smaller than normal (Exp. XXII, XXIII, XXIV). This change has already been noted by Santesson. The process, however, at least outside the body, may go much farther than this and rapidly result in complete laking of the blood (Exp. XXV and XXVI).

The adhesive and more or less gelatinous condition of the red corpuscles observed by Mitchell and Reichert in blood subjected to Crotalus venom has not been observed in experiments with Heloderma poison.

Clotting. — Numerous observers have found that the venom of many snakes delays or inhibits coagulation of the blood of animals into which it is injected, and Martin [1] has pointed out that the poison of the Australian Black Snake may cause thrombosis.

Experiments reported below (Nos. XXVIII, XXXIV, etc.) show that Heloderma poison, when injected into the vessels, may cause both these variations in the coagulability of the blood. Whether its injection ever results in serious thrombosis is not quite clear, but the finding of firm clots together with uncoagulable blood in the heart within a few minutes after this organ had stopped beating (Exp. XXVIII, XXIX, etc.) is strong evidence that it may do so.[2] The anomalous convulsions sometimes observed also find a ready explanation in thrombosis and embolism.

This diphasic influence upon coagulation is quite in accord with the observations of Lilienfeld upon the action of nucleohiston, which splits in the circulation into leuconuclein and histon, the former accelerating while the latter retards coagulation. One is tempted, therefore, to believe that the effects of *Heloderma* poison may be due to the same or similar substances, but whether these are derived directly from the venom or are set free by its cytolytic activity must, for the present, remain unknown. The researches of Delezenne,[3]

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[1] MARTIN: Journal and Proceedings of the Royal Society of New South Wales, 1895, xxix. pp. 146–276. See also Mitchell and Reichert, *loc. cit.*, p. 139.

[2] Van Denburgh found the blood in the hearts of pigeons firmly clotted while the auricles were still beating. *Loc. cit.*, p. 205, etc.

[3] DELEZENNE: Mécanisme d'action des substances anticoagulantes. Travaux de physiologie, 1898, pp. 284–320.

however, on the action of peptone, eel-serum, etc., seem to show that leuconuclein and histon are set free in the blood by destruction of the leucocytes, and that the leuconuclein later is removed by the liver while the histon remains in the blood and renders it incoagulable.

Experiment XXII, Mar. 20, 1900. — A drop of human blood and a drop of Heloderma poison were placed separately upon a cover-slip and were then mixed by pressing the cover-slip upon a slide. Within ten seconds all the red corpuscles appeared spherical. Later, the white corpuscles looked very coarsely granular and masses of granular matter accumulated about them. About three hours later, the serum was distinctly pigmented, but the red corpuscles were by no means decolorized.

Experiment XXIII, Mar. 20, 1900. — A drop of human blood was treated as in Experiment XXII, with the same results.

Experiment XXIV, Mar. 20, 1900. — The red corpuscles in a drop of human blood very quickly became spherical when treated with Heloderma poison.

Experiment XXV, Mar. 1, 1900. — Small dog. Killed by cutting cervical cord. Clotting time of blood taken from inferior vena cava after death varied from 2–3 minutes. Injected into left ventricle 2 minims fresh poison diluted with 3 minims normal salt solution. Thirteen minutes later took several cubic centimetres of bright scarlet blood from left ventricle.

Twenty-one minutes later this blood was still unclotted. It did not clot during the next twenty-four hours.

One hour and fourteen minutes after injection this blood appeared somewhat darker, and in less than an hour more was entirely laked.

Experiment XXVI, Nov. 6, 1899. — Six test tubes each received 10 cubic centimetres of a 0.9 per cent solution of sodium chloride and three drops of defibrinated dog's blood. Two of these tubes then received about a drop each of fresh poison, while two others each received a trace of poison. All the tubes were then kept at a temperature of 38°C. At the end of 4 min. and 20 sec. the blood in one of the strongly poisoned tubes was completely laked. At the end of fifteen minutes the salt solution in the other strongly poisoned tube was somewhat yellowish, while the remaining tubes showed no change. The experiment was then stopped.

Experiment XXVII, Apr. 20, 1900. — Six tubes each received a few cubic centimetres of a 0.6 per cent solution of sodium chloride and a drop or two of human blood. To each of two of these tubes was then added one drop of fresh undiluted poison, while two others received about one-sixth this amount and two were left unpoisoned for control. The tubes were poisoned at about 11.24

A. M. and were then kept at a temperature of about 37 C. until 3.45 P. M. The tubes were examined at frequent intervals, but showed no change until 12.15, when the blood in one of the strongly poisoned tubes seemed to be partially laked. At 1.45 the other strongly poisoned tube showed signs of laking. At 3.45 the blood in both strongly poisoned tubes was completely laked and the salt solution in the weakly poisoned tubes showed a faint trace of yellowish discoloration. The tubes were then kept at room temperature until the following morning when the controls showed no change and all the poisoned tubes showed complete laking.

Experiment XXVIII. Feb. 20, 1900. — Small mongrel dog. Five minims of fresh undiluted poison injected intravenously resulted in death in twenty-two minutes. Three minutes after the heart stopped beating, several cubic centimetres of liquid blood were taken from the right auricle. Five minutes later, the right ventricle was opened and found filled with liquid blood, while a firm yellowish clot, 10 mm. long, 2 mm. wide, rode on one of the trabeculae. The blood from the auricle did not clot within 48 hours.

Experiment XXIX. Feb. 13, 1900. — Small mongrel dog. Seven minutes after death the superior vena cava was cut and the blood pressed from the right auricle into a beaker. This blood was nearly all liquid, but contained a few firm clots. The right ventricle contained four clots of considerable size. No clotted blood was found elsewhere. The blood taken from the right auricle did not clot within 48 hours.

Experiment XXX. Nov. 23, 1899. — Small mongrel dog. Injected intravenously 24 minims of a solution of dried poison. Dead in ten minutes. Fourteen minutes after death, the auricles contained some small, firm, red clots, the right ventricle was nearly filled with a firm red clot, while the left ventricle was full of liquid blood in which were a few small clots.

Experiment XXXI. Feb. 6, 1900. — Medium sized bull-terrier. Intravenous injection of six drops of fresh poison in twenty minims of salt solution. Ten minutes after death the heart contained a number of fairly firm red clots, as well as a considerable amount of liquid blood.

Experiment XXXII. Nov. 1, 1899. — Dog. Injected intravenously twelve minims of fresh undiluted poison. Heart stopped thirteen and one-half minutes later. The right auricle was opened quickly and found to contain several firm clots, while the greater part of the blood was perfectly fluid.

Experiment XXXIII. Jan. 30, 1900. — Dog. Intravenous injection of six drops of fresh poison in fifteen minims of salt solution. Blood in heart was mostly fluid, but contained a few firm, dark red clots.

Experiment XXXIV, Nov. 15, 1899. — Mongrel fox-terrier. Samples of blood were taken in test tubes, from a cannula in the femoral artery, and the

clotting time observed by noting when the tubes could be inverted without causing the blood to flow. The cannula was carefully washed out with normal salt solution after each drawing of blood. Normal clotting time 3 min. 14 sec., 3 min. 58 sec., 3 min. 10 sec. Injected intravenously twenty minims of a weak solution of dried poison, and took clotting time at intervals during a period of one hour and a quarter.

	Min.	Sec.
First sample (2½ min. after injection)	1	20
Second "	1	40
Third "	1	44
Fourth "	1	47
Fifth "	3	04
Sixth "	9	58
Seventh "	14	00
Eighth "	15	20
Ninth "	13	20

Experiment XXXV, Nov. 4, 1899. — Dog. Clotting time taken as in Experiment XXXIV. Injected intravenously twenty minims of a strong solution of dried poison.

	Min.	Sec.
Normal clotting time	6–8	00
1 minute after injection, clotting time	5	28
3½ " " " " "	4	3
7½ " " " " "	5	50
12½ " " " " "	2	6
28 " " " " "	1	7
36½ " " " heart stopped		
37 " " " blood in heart unclotted.		

OTHER EFFECTS OF HELODERMA POISON.

Nervous System. — When Gila Monster poison is injected into the dorsal lymph sac of frogs it soon becomes apparent that the nervous system is profoundly affected. Usually the skin quickly becomes abnormally irritable, so that merely touching it with a thread causes the frog to make violent efforts to escape. This stage of hyper-irritability may, however, be entirely lacking. Its development probably depends upon the strength and quantity of poison absorbed. Soon the skin begins to be less irritable, and the loss of irritability continues until at last chemical, electrical, and mechanical stimuli fail to call forth any response. This increase of irritability and subsequent loss proceed from behind forward, so that the fore limbs

remain sensitive much longer than the hind limbs (Exp. XXXVII, XXXVIII). When one leg is protected by ligature from the poison injected into the dorsal lymph sac cutaneous irritability is lost as quickly in the protected leg as elsewhere (Exp. XXXVII, XXXVIII, XXXIX), and when the poison is confined, in the same way, to one leg there is no loss of irritability even at the point of injection (Exp. XL, XLI). The sensory paralysis, therefore, cannot be due to changes in the peripheral nerve-endings or fibres, but must be of central origin. Whether it is occasioned by changes in the ganglia or in the cord or both is not clear, but the fact that electrical stimulation of the posterior part of the spinal cord causes no contractions in the anterior limbs (Exp. XXXVIII) seems to show that afferent conduction in the spinal cord is seriously interfered with.

Great as are these changes in the sensory elements of the nervous system the motor elements seem to enjoy complete immunity. Santesson states that he found in frogs a curare-like action within two hours after injection, but his experiments are not convincing. Certainly in our experiments, with ordinary doses — in which death occurs in from one to four hours — no such effect has appeared. The irritability and conductivity of the motor cells, fibres, and end organs remain seemingly unaffected (Exp. XXXVI, XXXVII, XXXVIII). Whether this would continue true in cases of very slow poisoning, we have not attempted to show (see, however, Exp. XL), but it seems certain that a curare-like action plays no important part in Heloderma poisoning.

None of these changes has been made out in mammals.

Experiment XXXVI, Oct. 25, 1899. — Frog. Top of head was cut off to about the level of optic lobes. Retains sense of equilibrium, sits up and breathes and gives quite constant reflex when the toe is stimulated by dipping it into 0.15 per cent H_2SO_4. Rather large injection of dried poison dissolved in normal salt solution. After a few minutes the reflex became very slow and irregular. Soon the skin apparently lost all irritability and no reflex could be elicited with acid or pinching or pricking. Upon stimulation of the central end of the exposed sciatic with a strong alternating current, no movement was observable, but stimulation of the peripheral end caused normal contractions of the muscles of the leg. Electric stimulation of the cut end of the medulla caused violent contractions of the muscles generally. After all irritability in the skin had apparently been destroyed, the frog several times jumped, as much as a foot, without stimulation. The first visible effect of the poison was the almost immediate stopping of all respiratory movements.

Experiment XXXVII, Nov. 14, 1899. — Frog. A ligature was passed under the sciatic nerve and tied tightly around the right thigh. Response from this leg was somewhat less than from the left when the toes were stimulated.

2.00.25 P. M.   Two minims of moderately strong solution of fresh poison in normal saline were injected into the dorsal lymph sac.

2.02.45.   Slight hyper-irritability of skin of *both* legs.

2.05.   Skin a little less irritable.

2.07.   Skin of right leg a little less irritable than left.

2.14.   Both legs still respond to mechanical and electrical stimuli.

2.18.   The protected leg gives only slight response to stimulation but the left leg is still fairly irritable.

2.21.   Repeated injection, as before.

2.28.30.   Very much less irritable, the left leg draws away from the electrodes as if there were spinal reflex, while the right shows no reflex, but moves towards the electrodes because of tetanization of muscles.

2.40.   On stimulation of the skin over the gastrocnemius this muscle in the right leg gives much quicker response than in the left leg; the relaxation especially was quicker.

2.51.   No response to stimulation of either hind foot. Still draws fore foot away from stimulation and in trying to get away still uses hind legs. Later, the fore legs lost their irritability.

2.58.   Made tracings of the contraction curves of the gastrocnemius muscles of both legs. On stimulation both of the nerves and of the muscle substance the contraction of the muscle from the left leg was considerably weaker and longer than that of the muscle from the protected leg. This may or may not have been due to the action of the poison. No such effect has been observed in other experiments.

Experiment XXXVIII, Nov. 22, 1899. — Moderately large *Rana pipiens,* with right leg tied off from body by a ligature passed under the sciatic nerve.

3.05 P. M.   Seven minims of a weak solution of dried poison were injected into the dorsal lymph sac; and the dose was repeated at 3.14.

3.48.   Slight hyperæsthesia.

4.06.   Injected 1.5 mgm. of dried poison in 7 minims salt solution.

4.35.   20 minims weak solution of dried poison were injected.

4.40.   Still perfectly irritable everywhere.

4.50.   25 minims of the solution last injected were placed in the dorsal lymph sac.

4.59.   Appears rather torpid.

5.02.   Movement of hind legs still quick on stimulation.

5.04.   Movement of legs not so purposeful as hitherto.

5.08.   Toes of both hind legs still irritable, but not nearly as much so as earlier.

5.09.   No longer puts forefoot to tympanum when stimulated there.

5.10.   Right leg less irritable than left.

5.13.   Left leg gives very little response when the toes are stimulated.

5.15.   No response to strong stimulation (electric) of toes of either hind foot. Fore legs still irritable.

5.18.   No longer withdraws forefoot from electrode.

5.19.   Shows no sign of life.

5.21.   Heart exposed; still beating rhythmically. On opening dorsal lymph sac no discoloration was found.

5.28.   The spinal cord was exposed a short distance in front of the sacrum. When it was touched with the point of a knife, violent contractions of the muscles of the hind legs were observed.

5.31.   Heart still beating.

5.33.   Stimulation of the cord with the alternating current threw the hind legs into tetanus, but did not affect the fore limbs at all.

Experiment XXXIX. April 20, 1900. — Rana pipiens. Leg tied off as before.

11.39.   Injected about 1 minim of fresh venom in two of salt solution into dorsal lymph sac.

12.13.   Very quiet ; respiration intermittent.

1.00.   Very torpid; eyes closed ; no respiration except when roused ; skin still irritable.

2.00.   Same.

3.30.   Dead. Muscles of protected leg and of unprotected leg equally irritable when stimulated with minimal induction currents both directly and through the nerves.

4.15.   Sinus venosus responds to stimulation, but ventricle cannot be made to beat.

4.30.   Blood corpuscles not changed in shape.

Experiment XL. November 23, 1899. — Frog. Right leg tied off with a ligature passed under the sciatic nerve.

10.59 A. M. Two minims of fresh undiluted poison were injected into the right leg below the ligature.

12.00.   No change is apparent.

1.00.   Same. The skin at the point of injection is as irritable as elsewhere.

Nov. 24. — 10.36 A.M. Skin of both legs perfectly irritable. Muscles of both legs respond well to very weak electrical stimulation of nerves and of muscle substance. Removed ligature.

11.50.   Little change.

Nov. 25. — Found dead.

Experiment XLI. April 20, 1900. — *Rana clamata.*   Right leg tied off as before.

11.09.   Three minims fresh undiluted poison were injected into the right leg below the ligature.

11.14.   Leg perfectly irritable and muscles functional.

11.27.   Same.

11.31.   Same.

12.12.   No change.

2.00.   No change.

4.30.   Still no apparent change.

Apr. 21. — 10.15 A. M. — Leg much discolored.   Muscles cannot be made to contract either by direct stimulation or by stimulation of nerves.   Skin of poisoned leg seems slightly irritable.   Frog, aside from this leg, perfectly normal.

10.30.   Removed ligature.

12.10.   Dead.

Voluntary Muscle. — The only evidence that Heloderma poison may act upon muscles is recorded in Experiments XXXVII and XLI. It is, perhaps, probable that the slowness of relaxation and muscle death found in these two experiments were not due to any action of the poison.   Thus in the latter experiment the death of the muscles might well have been caused by the ligature alone.

The Secretion and Expulsion of Urine. — Experiments one, two, and three show that frequent micturition is a quite constant symptom when Heloderma poison is injected into unanæsthetized dogs.   It is commonly observed also in poisoned dogs when under the influence of ether.   It occurs also in rabbits, but has been noted only once in experimenting with cats.   Experiment XLII indicates that the explanation of this sometimes nearly continuous micturition is to be found in a slow contraction of the bladder.   Whether this is due to direct action of the poison on the muscular tissue of the bladder or to nervous effects has not been shown.

As might have been predicted from the great fall in blood-pressure, the secretion of urine is almost immediately stopped.

Experiment XLII. Mar. 15, 1900. — Small dog.   Cannulas in the ureters.

4.55 — 5.05 P. M.   Secreted 3.4 c.c. urine.

5.06.   Injected 10 minims of a solution of dried poison.

5.06 — 5.16.   Secreted 0.1 c.c. urine.   During this time the bladder began to contract slowly, causing a gradual expulsion of urine.   This continued until about 5.20, when the bladder was hard and firmly contracted — a condition in which it remained until after death.   No contraction of the intestines was observed.

Post-Mortem Findings.

Post-mortem examinations of the tissues of most of the animals experimented upon have been made, and at one time or another sections of all the important organs have been cut. With one exception (Exp. I), in which there was slight œdema and very slight discoloration about the point of injection, no local changes have been observed in dogs, cats, or frogs. In three or four instances subendocardial extravasations of small extent have been found on the left side of the ventricular septum, but no extravasations have been observed in other organs. The right side of the heart and the whole venous system, except in the lungs, are greatly distended with blood. The venous congestion in the intestines and kidneys, sometimes also in the bladder and spleen, is enormous. The changes in the blood itself have already been described.

Conclusions.

In conclusion, it may be well to summarize briefly the more important effects of Heloderma poison as deduced from our experiments.

1. The effects of Gila Monster poison differ in no *important* respect from those of various snake venoms.

2. The poison appears to act directly upon the respiratory centre, causing a quickening and then a gradual paralysis of respiration.

3. The heart also exhibits a period of increased activity followed by gradual paralysis. These cardiac effects are probably due to local action of the poison.

4. The vasomotor centre shows no evidence of primary stimulation, but injection is immediately followed by a great fall in blood-pressure.

5. The great primary fall in arterial pressure is due to vascular dilatation — the central or peripheral origin of which has not been clearly shown. The gradual secondary fall is caused by cardiac failure.

6. The motor nerves, with their cells and end organs, remain entirely unaffected.

7. The sensory apparatus suffers an increase in irritability, followed by a total loss. These changes proceed from behind forward, and are of central origin.

8. Coagulation of the blood is at first accelerated, then retarded.

Serious thrombosis may, doubtless, occur.   The blood may be rendered incoagulable.

9. The red corpuscles are often caused to become spherical, and the blood, at least outside the body, may be laked.

10. Death usually results from paralysis of the respiratory centres, but when artificial respiration is maintained death supervenes as the result of cardiac failure.   Thrombosis must be regarded as a *possible* cause of death.

11. The secretion of urine is stopped.   Frequent micturition is caused by the slow contraction of the bladder.

12. Œdema and slight extravasation are sometimes, though very rarely, caused by Heloderma venom.

A STUDY OF THE EFFECTS OF COMPLETE REMOVAL OF THE MAMMARY GLANDS IN RELATIONSHIP TO LACTOSE FORMATION.

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IT is at present universally assumed without any adequate experimental proof that the sugar of milk is formed in the cells of the mammary gland. This assumption is made mainly because that sugar is characteristic of the period of lactation and is not found elsewhere in nature than in connection with lactation. During lactation and, according to some observers, during the latter part of the period of gestation, lactose is also found in the urine, a certain proof that it exists in the blood. And it has further been assumed, again without experimental evidence, that this lactose of the blood and urine arises from a leakage from the charged cells of the mammary gland. Now, until some experimental evidence has been acquired on the subject it is obviously just as feasible to suppose that the lactose is formed elsewhere in the body, and merely secreted by the mammary gland. On such an hypothesis the presence of lactose in the urine during lactation would be explained by supposing that a small fraction of the lactose formed elsewhere and poured into the blood for secretion by the mammary glands was removed by the kidney cells. Further, but little experimental work has been done upon the formation of lactose in the body. Even if it be admitted that lactose is formed in the mammary gland, from what materials it is there formed is entirely unknown; whether it is formed, for example, directly from dextrose or some other carbohydrate precursor absorbed by the cells from the circulating blood, or whether it is formed from carbohydrate stored up temporarily by the cells in a form comparable to the glycogen of the liver cells.

Some light can be shed upon these problems by a study of the condition of the urine at the time of parturition in the absence of the mammary glands. The act of parturition is the signal for the commencement of active secretion of milk, and if the lactose be formed elsewhere to be thrown out in the milk, there should be in all probability a temporary appearance of lactose in the blood, and hence in

the urine, near the time of parturition even in the absence of the
mammary gland. Since in this case the lactose would not be re-
moved in the normal manner by the mammary gland its formation
would rapidly decrease and finally the lactose would disappear.
Again, even if lactose be formed in the mammary gland, it is possible
that such an experiment as is above described might indicate some
carbohydrate precursor of lactose, which is normally produced in
small percentage and carried in the blood to be transformed in the
cells of the mammary gland to lactose. Such a precursor of lactose
formed elsewhere than in the gland would also tend at first to be
formed at parturition even in the absence of the gland, and under
such conditions might accumulate sufficiently in the blood to be
thrown out by the kidneys and be recognized in the urine.

On the other hand, a negative result to such an experiment would
indicate in the first place that lactose is formed in the mammary gland,
since it is completely absent in the absence of the gland; and,
secondly, that the whole process of lactose formation takes place in
the gland, since, in the absence of the gland, no intermediate products
appear elsewhere in the body.

A few experiments on the effects of removal of the mammary glands
on lactose formation were carried out by Paul Bert;[1] and the present
experiments originated in a suggestion by Schäfer,[2] in an article on
the mechanism of the secretion of milk, that these experiments of
Bert deserved repetition.

The experiments can only be satisfactorily performed on a fairly
large animal with a small number of glands. Since the period of
gestation is considerable, a large number of experiments by the same
observers is made almost impossible because of the expense and time
involved.

Bert made his first experiment upon a guinea pig, with negative
but somewhat unsatisfactory results. After searching in vain for
some substance which would yield lactose in appreciable quantity in
the tissue of the mammary gland and hence prove the formation of
lactose in the gland, he next performed the operation of complete
removal of the glands in two goats. In both animals no reducing
substance was found in the urine throughout the entire period of
gestation; and in both a ready reduction of alkaline copper sulphate
solution was obtained for some days after parturition. The amount

[1] BERT: Comptes rendus de l'Académie des Sciences. Paris. 1884. xcviii, p. 775.
[2] SCHÄFER: Textbook of physiology, 1898. i, p. 665.

of reduction was greatest immediately after parturition, steadily decreased each day, and in the more pronounced of the two experiments had practically disappeared on the ninth day after the birth of the kid. Bert does not appear to have made any quantitative experiments as to the amount of reducing substance present, nor to have identified in any way the substance giving the reduction.

Our experiments were performed on two kids, which were kindly presented to us for the purpose by Prof. Graham Lusk. The animals were received in the laboratory when about a fortnight old and were fed until able to eat fodder, when they were farmed out.

The urine was examined at intervals to obtain an estimate of the normal reducing power of goat's urine. It was found that the urine gave a *feeble* reduction with both Fehling's and Almen's test, particularly the latter, but nothing to indicate an appreciable amount of a reducing sugar.

The animals were served at about the same time. Gestation was allowed to proceed for a considerable period[1] in order to permit such an increase in the size of the glands as would ensure their complete removal, and then in each case the mammary glands were completely extirpated.

A median longitudinal incision was made between the teats, and the glands were carefully dissected, first from the skin, with a cut across the ducts at each teat, and afterwards from the underlying tissue. In each case the gland tissue was compact and could be easily removed entire.

The animals recovered rapidly and completely from the operation and remained in perfect health throughout the remainder of the experiment.[2] Both before the operation and throughout the remainder of the period of gestation the urine was carefully tested for reducing sugar and also for other abnormal constituents and was found in every respect normal.

Parturition took place in both cases under normal conditions, and the urine was examined for several days afterwards. In the case of the first animal no change whatever in the urine was observed, there was no increase in reducing power, and there was complete absence of albumen. In the second case a definite but slight increase in

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[1] For eight weeks in one case, and thirteen in the other: the period of gestation is about twenty-one weeks.

[2] It is interesting to observe that the teats in both cases went on developing after removal of the glands and attained a normal size.

reducing power for Fehling's solution was observed in the urine col-
lected during the two days after parturition, an increase which dis-
appeared on the third day and did not reappear. The reduction,
even when the copper sulphate was not present in excess, was not
complete; instead of the usual yellowish-red given by a reducing
sugar, a brown color was obtained, similar to that in the normal urine,
only considerably more pronounced. Hence it was impossible to
estimate with any accuracy by Fehling's solution the amount of
reducing substance, calculated as dextrose, present in the urine. The
amount of reduction was not increased by previous boiling with a
mineral acid followed by neutralization, showing the absence of
lactose. Thus, a determination before boiling with acid showed a
disappearance of blue color which indicated an equivalent of 0.37 per
cent of dextrose; while after boiling for half an hour with 25 per cent
of strong hydrochloric acid, neutralizing, and again determining, 0.36
per cent was obtained. These figures are quoted to show that there
was no increase in reduction such as lactose if present would have
given, and not as proving that any such amount of a reducing sugar
as they indicate was present. The readings were taken when the
Fehling's solution was completely decolorized, but the reduction was
very incomplete, giving a dark brown precipitate containing much
unreduced copper oxide. In our opinion, no reducing *sugar* was
present, but only traces of a substance of feeble reducing power. We
also attempted by the usual methods to obtain crystals by the phenyl-
hydrazine test, but with negative results. Further, addition of small
percentages of dextrose or lactose to the urine gave the usual charac-
teristic type of reduction with Fehling's test.

Our results are accordingly opposed to those of Bert in that we
obtain no appreciable increase in reducing power in the urine at the
time of parturition after removal of the mammary glands, such as
would indicate the presence either of lactose, or any carbohydrate
precursor of lactose, formed elsewhere than in the mammary gland.

We accordingly believe that lactose is formed in the cells of the
mammary gland, and that the complete process of formation takes
place in the gland and not from any intermediate substance carried
to the gland by the blood. Further, there is no increased percent-
age of dextrose in the blood at parturition such as, in the absence
of the gland, would lead to the appearance of dextrose in the urine.
Also, the lactose which appears in the urine during lactation must
arise from absorption from the charged gland cells.

BRIEF CONTRIBUTIONS TO PHYSIOLOGICAL CHEMISTRY.

COMMUNICATED BY LAFAYETTE B. MENDEL.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

CONTENTS.

I.

ON THE OCCURRENCE OF IODINE IN CORALS.

BY LAFAYETTE B. MENDEL.

BAUMANN'S discovery of iodine as a normal constituent of the animal body, and Drechsel's investigations on the iodine compounds of a Mediterranean coral have served to direct attention again to the physiological rôle of this element.[1] Drechsel demonstrated that the horny axial skeleton of Gorgonia cavolinii which he obtained at Naples contains iodine in organic combination. This skeletal substance, termed *gorgonin* by him, yielded on decomposition with baryta water a well crystallized iodo-amido acid, corresponding in composition with a moniodo-amidobutyric acid. Drechsel did not regard the latter as a distinct component of the gorgonin, but assumed it to be a characteristic cleavage product of the complex iodine-containing, keratin-like albuminoid of the coral skeleton. The further peculiar fact that the cœnenchyma of the animal contains practically no iodine, led Drechsel to the interesting conclusion that Gorgonia cavolinii has a specific iodine metabolism which is essential to the building up of the framework of the axial skeleton. Hundeshagen[2] had previously investigated an iodine-containing organic substance which he separated from marine sponges; and later Harnack[3] isolated from the same source a compound which he has

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[1] Cf. BAUMANN: Zeitschrift für physiologische Chemie, 1896, xxi. p. 319. DRECHSEL: Zeitschrift für Biologie, 1896, xxxiii, p. 90.

[2] HUNDESHAGEN: Zeitschrift für angewandte Chemie, 1895, p. 473.

[3] HARNACK: Zeitschrift für physiologische Chemie, 1898, xxiv, p. 412.

termed *iodospongin*, containing on an average 8.2 per cent of iodine. Other investigators[1] also have more recently demonstrated the presence of iodine in organic combination in other marine organisms.

Through the kindness of Professor Verrill I have had an opportunity to examine specimens of three species of corals which were collected in the West Indies. These species,[2] Gorgonia flabellum, Gorgonia acerosa, and Plexaura flexuosa, resemble Gorgonia cavolini in many respects. The latter is, however, distinctly a Mediterranean species, while the others have been found in the West Indies only. Gorgonia flabellum grows to a large size; it is flabellate, and throughout finely reticulate. The fronds are sometimes two feet high and nearly as broad. The color varies from an ash to a bright yellow, and is occasionally red. The polyps are everywhere scattered, except where the wing-like processes commence to grow from the surface, and in that case they become lateral. Gorgonia acerosa is the large, purple, pendulous species of the West Indies. When young, the branchlets are erect or nearly so, and the pinnate character is less distinct than in adult specimens. The latter are very large, often five feet high. The axis is black. There are either one or two rows of polyps on the opposite sides of the branchlets. Plexaura flexuosa has a fulvous or purplish color. The branches are terete and without verrucæ, but have a slightly and minutely uneven surface, owing to the fact that the oscules are either situated in a slight depression of the cortex, or have the inferior side a little prominent. The length of the branchlets is often six inches.

In each of these species iodine was found to be a constituent of the horny axial skeleton. The organic material was fused with pure sodium hydroxide and potassium nitrate, and the fusion products were tested for iodine in the usual way by acidification and extraction with chloroform. All the reagents used were previously ascertained to be absolutely free from iodine. In order to afford a quantitative comparison with the specimens examined by Drechsel, the adherent material was carefully separated from portions of the air-dry axial skeletons, and the latter comminuted and dried for analysis at 110° C. After fusion in a nickel crucible with pure sodium hydroxide, the halogens were precipitated as silver salts and determined by the method which Drechsel[3] employed. In

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[1] ESCHLE: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 30. GAUTIER: Comptes rendus de l'Académie des Sciences, 1899, cxxviii, p. 1069.

[2] See DANA: Wilkes Exploring Expedition, vii, Zoöphytes, pp. 650, 665, 668.

[3] DRECHSEL: Zeitschrift für Biologie, 1896, xxxiii, p. 96.

the table of results, the analyses of Gorgonia cavolinii are added for comparison.[1]

SUMMARY OF THE ANALYSES.

Species.	Loss of weight at 110° C. Per cent.	Iodine. Per cent.	Chlorine. Per cent.
Gorgonia acerosa.	13.38	1.70	3.17
Gorgonia flabellum (average).	10.44	1.15	1.24
Plexaura flexuosa (average).	13.40	0.28	0.86
Gorgonia cavolinii (Drechsel).	11.16	7.79	2.18

While the results presented are considerably smaller than the figures obtained for Gorgonia cavolinii, they compare more closely with the published analyses of certain algæ[2] (Fucus vesiculosus, Laminaria digitata) and with Harnack's determinations of the iodine content (1.5 per cent) of ordinary sponges.[3] It is suggestive to note that the species of West Indian coral richest in iodine is the one most closely related to the Mediterranean Gorgonia cavolinii.

In his monograph on "The physiological rôle of mineral nutrients," Loew[4] has called attention to the possibility of the presence of bromine in conjunction with the iodine and chlorine in the Gorgonias. With a view to ascertaining this point, I have examined very carefully a relatively large quantity (30 grams) of the skeleton substance of Gorgonia acerosa. The latter was selected because it was the richest in halogens of all the species examined. The results were entirely negative; not a trace of bromine was found. Lastly, the skeleton substance of Gorgonia flabellum was decomposed (in quantities of 50 to 75 grams) with baryta water. The method pursued by Drechsel in isolating his compound $C_4H_5NIO_2$ was closely followed, but without success. Solutions containing organic iodine compounds were obtained, but no corresponding amido-acid could be isolated. Whether this result is due to the relatively small quantities

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[1] Most of these analyses were made by A. N. Richards, B. A.

[2] ESCHLE : *loc. cit.*

[3] HARNACK : *loc. cit.* p. 414.

[4] LOEW : U. S. Department of Agriculture : Division of vegetable physiology and pathology, 1899, Bulletin No. 18, p. 21. footnote.

of the material available,[1] or to a difference in the way in which the iodine exists in combination in these species cannot be definitely stated.

The preceding observations afford further justification for the belief already maintained by Drechsel, that for many organisms iodine is as essential an element as is chlorine for others; and that in the absence of iodine the normal nutrition of the organism may be interfered with. Without some assumption of this nature, it is difficult to understand why organisms like the Gorgonias should store up in their horny axial skeleton an element existing only in traces in sea water, and apparently not entering into the constitution of the true growing cœnenchyma of the animal.

II.

GLYCOGEN FORMATION AFTER INULIN FEEDING.

By R. NAKASEKO.

INTRODUCTORY.

THE carbohydrate inulin, which readily breaks down to levulose by hydrolysis with acids, has long been suggested as a suitable foodstuff in those forms of diabetes in which levulose can be utilized.[2]   Sandmeyer,[3] however, found that inulin was very poorly utilized in diabetic dogs, a large part of the ingested carbohydrate being eliminated again unchanged in the fæces. In view of the marked effect which levulose, in common with many other carbohydrates, exerts in increasing the glycogen-content of the liver, it was natural to look for a similar result in the case of inulin-feeding.   To test this possibility, Miura[4] carried out a series of experiments on rabbits in Külz's laboratory several years ago.   Precaution was taken to procure pure inulin for feeding, and the glycogen of the liver was estimated by the Brücke-

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[1] DRECHSEL obtained only 0.34 gram of the pure amido-acid from fifty grams of gorgonin.

[2] KÜLZ : Beiträge zur Pathologie und Therapie des Diabetes Mellitus. Marburg, 1874.

[3] SANDMEYER : Zeitschrift für Biologie, 1894, xxxi, p. 32.

[4] MIURA : Zeitschrift für Biologie, 1895, xxxii, p. 255.   The references to the older literature on inulin-feeding are given in this paper.

Külz method. The rabbits had all been starved for six days to reduce
the store of glycogen in the liver to a minimum; thereupon the inulin
was administered in small doses at hourly or half-hourly intervals, in
order to offer the most favorable conditions for inversion and absorp-
tion. The total quantity of carbohydrate ingested varied from ten to
twenty-five grams per animal, and the rabbits were killed ten or twelve
hours after the administration of the last dose. As a basis for com-
parison Miura took the glycogen determinations made by Külz [1] on
fasting rabbits. Külz found as a maximum for the glycogen-content
of the liver after six days fasting 0.9 per cent of glycogen, the equiva-
lent of 0.329 gram of glycogen, or 0.252 gram per kilo of body weight.
In nineteen experiments with inulin-feeding as outlined, the glycogen-
content of the liver was increased in the majority of cases; in six
animals, however, negative results were obtained. Of the thirty-six
experiments by six different investigators on inulin-feeding and gly-
cogen-formation, which we have found recorded in physiological
literature, nineteen have given negative results. The experiments of
Miura are the most careful and satisfactory of all in point of technique.
He concludes his research with the following remarks: " Renewed
investigation is demanded to determine whether the levulose found
(post-mortem) in the various portions of the intestine owes its forma-
tion to the action of one or more of the digestive juices, to the acid
of the stomach, or to vegetable enzymes derived from the food
ingested. In view of the marked increase in the glycogen-content
of the liver which follows levulose feeding, the conclusion is inevi-
table that ingested inulin is either converted to levulose only in part,
or too slowly to permit any storing up of glycogen from the quanti-
ties of sugar absorbed. Herein, perhaps, lies the explanation of
the inconstancy of the experimental results." [2]

Regarding the behavior of inulin towards amylolytic enzymes, the
experiments of Mr. A. B. Siviter [3] in this laboratory afford an answer.
He found that the ordinary amylolytic enzymes such as the ptyalin
of saliva, the amylopsin of the pancreatic extract, vegetable diastase
and "Taka" diastase — a very active enzyme preparation obtained

[1] KÜLZ: Festschrift für C. Ludwig, 1890, p. 69; Centralblatt für Physiologie,
1890, iv, p. 788.

[2] MIURA: Zeitschrift für Biologie, 1895, xxxii, p. 265.

[3] See CHITTENDEN: This journal, 1898, ii, p. xvii. Cf. also the recent papers
by RICHAUD: Comptes rendus de la société de biologie, 1900, lii, p. 416. BIERI
and PORTIER: *ibid*, 1900, lii, p. 423.

from Eurotium oryzæ — are without action on inulin. Dilute hydrochloric acid (0.05–0.2 per cent) at 40° C. transforms inulin to levulose. Hydrochloric acid combined with proteids likewise inverts inulin to levulose, but more slowly than corresponding strengths of *free* acid. Organic acids, such as oxalic, lactic, and salicylic, also transform inulin in the same way.

In adding a number of new experiments on inulin-feeding to those already referred to, we have also had in mind the negative, yet inconclusive experiments carried out in this laboratory with the resistant carbohydrate lichenin.[1] We have desired especially to supplement Miura's investigation by administering large portions of inulin and allowing the absorption to proceed during longer intervals than has heretofore been the case. It was hoped to counteract in this way the effects of a possible *slow* conversion and absorption of the carbohydrate. It is difficult, however, to determine upon any satisfactory time-limit, owing to the danger of a subsequent loss of the newly stored-up glycogen during the protracted hunger period.

EXPERIMENTAL.

In these experiments we have followed Miura's method with few variations. The data thus afforded are summarized in the table below. The inulin used was obtained from various sources and was purified with alcohol, when necessary, until it gave no reduction with Fehling's solution after being heated three minutes in a boiling water-bath.[2] It was free from nitrogenous matter. The rabbits were starved from five to seven days. Inulin was suspended in warm water and administered, partly dissolved, with a stomach sound. The animals were killed by decapitation; the liver was quickly removed, and the glycogen determined by the Brücke-Külz method. The stomach and intestinal contents were tested with Fehling's solution and by Seliwanoff's reaction (HCl and resorcin) for levulose or inulin, other sugars which give the reaction, such as saccharose, being assumed to be absent after long fasting. The observations on these reactions are not recorded here, since they afford no new points of interest. Other details are included in the table, and two experiments with levulose are added for comparison.

[1] Cf. BROWN: This journal, 1898, i, p. 458.
[2] As advised by Kiliani. Cf. MIURA: *loc. cit.*, p. 260, note 1.

		Inulin feeding.							Levulose feeding.	
No. of experiment.		1	2	3	4	5	6	7	8	9
Initial body-weight.	Grams.	1650	1925	1925	1475	2575	2200	2120	2675	2450
Final body-weight.	Grams.	1575	1750	1500	1260	2575	1920	1820	1640	2450
Duration of fasting.	Days.	7	7	6	5½	5½	7	5½	5	0
Amount of substance fed at each dose.	Grams.	2	2	2	4	4+8	2+8	4+8	4+8	4+12
Total quantity of carbohydrate fed.	Grams.	18	20	20	26	23	25	33	32	16
Interval between first and last feeding.	Hours.	16	12	12	26	48	41	48	30	2½
Interval between last feeding and death.	Hours.	5	10½	12	5	12	5	15	17	15
Weight of the liver.	Grams.	56	53	46	33	52	39	38	42	88
Total glycogen found.	Grams.	0.0223	0.0518	0.2609	0.4646	0.5067	0.7555	0.2411	2.0640	4.6433
Percentage of glycogen in the liver.	Per cent.	0.06	0.20	0.51	1.23	0.98	1.94	0.64	4.91	5.28
Glycogen per kilo of final body-weight.	Grams.	0.006	0.030	0.129	0.323	0.224	0.360	0.133	1.259	1.895

CONCLUSION.

A study of the figures presented reveals the fact that an increase of glycogen in the liver above the starvation maximum (0.9 per cent, or 0.252 gram per kilo) ascertained by Külz was obtained in only three cases after inulin-feeding (experiments 4, 5 [?], 6). Any connection between the glycogen-content and the length of time during which the feeding continued is not evident from the data obtained. The well known glycogen-forming property of levulose is again demonstrated in the last experiments and stands in striking contrast to the practically negative results with comparable quantities of inulin. Furthermore, we recall the statement of Kulz [1] — an authority on glycogen-formation — that occasionally there are to be found in the liver of the rabbit quantities of glycogen presumably too large to disappear to the extent recorded in the usual experiments, even after six days' fasting. In addition to this, the stomach of the fasting rabbit normally always contains particles of food residue which may gradually offer available carbohydrate.

In view of all these facts, which apply equally to Miura's experiments and to our own, the glycogen-forming properties of inulin, in the case of the rabbit at least, must still be regarded as uncertain or minimal. [2]

III.

THE INFLUENCE OF ACIDS ON THE AMYLOLYTIC ACTION OF SALIVA.

BY G. A. HANFORD.

INTRODUCTORY.

A SERIES of experiments on the influence of acids and alkalies upon the amylolytic action of the saliva has recently been undertaken by F. Kübel [3] in Professor Grützner's laboratory. The results obtained are largely confirmatory of investigations carried out long

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[1] KÜLZ : Centralblatt für Physiologie, 1890, iv, p. 789.

[2] Cf. also RICHAUD : Comptes rendus de la société de biologie, 1900, lii, p. 416.

[3] KÜBEL : Archiv für die gesammte Physiologie, 1899, lxxvi, p. 276.

ago in this laboratory by Chittenden and Smith.[1] As Kubel was apparently not familiar with this work, and inasmuch as other recent writers[2] have overlooked some of the results long established, it seemed desirable to review the older observations here very briefly and to add some experiments of our own which may serve to explain and extend the work of Kübel.

From careful quantitative experiments, in which the extent of amylolytic action was determined by a direct estimation of the sugar formed, Chittenden and Smith concluded as follows:—

"The most favorable condition for the diastatic action of ptyalin, under most circumstances, appears to be a neutral condition of the fluid together with the presence of more or less proteid matter. The addition of very small amounts of hydrochloric acid, however, to *dilute* solutions of saliva, giving thereby a *small percentage* of acid-proteids, appears to still further increase diastatic action. Under *such conditions* a minute trace of free acid appears to still further increase the action.

"0.003 per cent free hydrochloric acid almost completely stops the amylolytic action of ptyalin. The larger the amount of saturated proteids, the more pronounced becomes the retarding action of free acids.

"The retarding effects of smaller percentages of free acid are not due wholly to destruction of the ferment. Pronounced destruction takes place with 0.005–0.01 per cent free hydrochloric acid.

"Proteid matter, in influencing the diastatic activity of salivary ptyalin acts not only by combining with acids and alkalies, but apparently also by direct stimulation of the ferment."[3]

Kubel employed a method introduced by Paschutin for the estimation of relative amylolytic activity: at the end of each trial digestion equal quantities of potassium hydroxide solution were added to the digestion mixtures, and the whole was then plunged into a boiling water-bath for a definite interval. In order to estimate more accurately the extent of digestive action from the intensity of the yellow-brown color brought about by the reaction of the caustic

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[1] CHITTENDEN and SMITH: Studies from the laboratory of physiological chemistry, Yale University, 1885, i, p. 1. Also transactions of the Connecticut Academy, 1885, vi, p. 343: Jahresbericht für Thierchemie, 1885, xv, p. 256.

[2] *E.g.* AUSTIN: Boston medical and surgical journal, 1899, cxl, p. 325.

[3] CHITTENDEN and SMITH: Studies from the laboratory of physiological chemistry, Yale University, 1885, i, p. 33.

alkali with the soluble products of amylolysis (Moore's reaction), Kübel devised a colorimetric procedure suggested by that of Grutzner for pepsin determinations. Solutions of potassium bichromate in various definite dilutions were employed for the color comparisons. Although a method of this character is less exact than direct quantitative determinations of the sugar formed, it gives quite comparable results and makes it possible to carry out a very large number of estimations at one time. From such experiments Kübel concluded that for a two per cent starch paste, acids of $\frac{1}{500}$ n or weaker strengths considerably facilitate the amylolytic action of human mixed saliva.[1] Greater concentration of acid tends to inhibit the amylolytic power, the degree of inhibition varying, for equimolecular strengths, with the acids used.

A comparison of Kübel's work with the earlier investigations from this laboratory makes it evident that his conclusions have for the most part been anticipated. Thus Chittenden and Smith clearly demonstrated that small percentages of acid-proteids tend to increase amylolytic action, and that a minute trace of *free* acid appears to facilitate the action still further. Kübel's results, while they are comparable with one another, will not permit any general conclusion such as he has drawn regarding the *exact percentages* of acids or alkalies which stimulate or inhibit amylolytic activity; for the work of previous investigators has emphasized the important influence which the presence of proteids may exercise — a fact apparently overlooked by Kübel. Proteid matter tends to prevent the destructive or inhibitory influence of acids by combining with it; in comparing the rate of digestive action under different conditions, it is obviously necessary to take into consideration the amount of proteid present. Thus the dilution of the saliva, or the differences in the concentration of the secretion collected at different times or from different individuals may bring about digestive variations when all the other conditions are constant, owing to the varying quantities of proteid thus introduced. Further progress was made when it was demonstrated by Chittenden and Smith that *free* acid, *i. e.* acid which reacts with tropæolin ∞, readily destroys the enzyme of the saliva. The difference between free and combined acid in their relative destructive or inhibitory power appears to have been overlooked by Kübel. It will be shown in the experiments to follow

[1] KÜBEL: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 303

that digestion never proceeds at all in the presence of more than the merest traces of *free* hydrochloric acid; and it would seem probable from our repetition of Kübel's experiments that in those instances in which he observed complete inhibition of digestive action, *free* acid was usually present. The conception of *free* acid as an inhibitory agent towards ptyalin carries with it a definite idea; it is independent of any consideration of the accompanying conditions, such as amount of saliva used, concentration of saliva or strength of starch paste. Whenever the digestive mixture gives a reaction for *free* acid, no amylolysis is to be expected. On the other hand, to speak of a definite total acidity of the fluid as influencing amylolysis in a definite way is scarcely permissible without defining carefully the conditions of the reaction, in particular the amount of proteid present. Even the different proteids vary widely in their combining power with acids.

EXPERIMENTAL.

In the part following we shall attempt to demonstrate some of the points just discussed, by extracts from the protocols of our experiments. Our method has been similar to that of Kübel. Starch paste made from pure, neutral, arrowroot starch, acid of known strength, and lastly saliva variously diluted, have been mixed together, and the extent of amylolysis at different temperatures determined after various intervals (usually ten minutes). Kübel's colorimetric method was followed in estimating the relative rate of digestion. The presence of *free* hydrochloric acid was tested for by the delicate reaction with dimethyl amidoazobenzol first introduced by Töpfer.[1]

First Series. — The experiments of this series illustrate how the individual conditions may influence the limits of amylolytic action independently of the quantitative relations of the reagents which enter into the digestive mixture. They are intended to contrast with Experiment 19 of Kübel. As in his experiment, so here, a four per cent neutral starch paste was used; mixed human saliva was diluted with one part of water, filtered, and finally diluted with three parts of glycerine. Different lots of saliva were employed. Under such conditions Kübel found — with his own saliva and wheat-starch paste — that in a mixture containing five c.c. of starch paste, 0.3 c.c. of saliva, and five c.c. of acid of known strength, the diges-

[1] TÖPFER : Zeitschrift für physiologische Chemie, 1894, xix, p. 104.

TABLE A.

Temperature, 17° C. Period of Digestion, 10 minutes.

Starch paste. c.c.	Diluted saliva. c.c.	HCl added. c.c.	Resultant acidity as HCl. Per cent.	Experiment I.		Experiment II.		Experiment III.	
				Reaction for *free* HCl.	Results.	Reaction for *free* HCl.	Results.	Reaction for *free* HCl.	Results.
5	0.3	5 H$_2$O	0	—	Digestion.	—	Digestion.	—	Digestion.
5	0.3	5 $\frac{N}{1000}$	0.0036	—	No digestion.	—	Digestion.	—	No digestion.
5	0.3	5 $\frac{N}{500}$	0.0072	+	No digestion.	—	No digestion.	—	No digestion.
5	0.3	5 $\frac{N}{200}$	0.0182	·	No digestion.	+	No digestion.	+	No digestion.

tion at room temperature and during a period of ten minutes was facilitated by hydrochloric acid of $\frac{n}{2000}$ to $\frac{n}{400}$ n resultant strength, and entirely checked by $\frac{n}{200}$ n or stronger hydrochloric acid. In the typical protocols tabulated (Tables A, B) it will be seen how the limits of digestive action may vary with saliva from different sources, etc. It will also be noted that *digestion has not proceeded* in those experiments in which *free* hydrochloric acid was detected.

TABLE B.

In these experiments each test tube contained 5 c.c. starch paste, 0.3 c.c. dilute saliva, 5 c.c. HCl of the strength indicated. Temperature, 17° C. Period of digestion, 10 minutes.

No. of Tube.	HCl added. c.c.	Total acidity as HCl. Per cent.	Reaction for *free* HCl.	Results.[1]		
				Experiment I.	Exp. II.	Exp. III.
1	5 H_2O	0	−	Digestion, 5	Digestion, 5	Digestion, $2\frac{1}{2}$
2	5 $\frac{n}{1000}$	0.0036	+	No digestion.	No digestion	No digestion
3	5 $\frac{n}{2000}$	0.0018	−	Digestion, 5	Digestion, 2	Digestion, $2\frac{1}{2}$

[1] The figures refer to the artificial color scale indicating relative extent of amylolysis.

The favorable action of very small quantities of acids (*combined* acid in every case) is demonstrated, in agreement with previous observers, by the following protocol: —

TABLE C.

Period of digestion, 10 minutes. Temperature, 37° C.

No. of Tube.	Diluted saliva added. c.c.	HCl added. c.c.	Total acidity as HCl. Per cent.	Reaction for *free* HCl.	Result.
1	0.3	5 H_2O	0	−	Digestion, 2
2	0.3	5 $\frac{n}{800}$	0.0043	−	No digestion
3	0.3	5 $\frac{n}{1600}$	0.0021	−	Digestion, 5
4	0.3	5 $\frac{n}{3200}$	0.0010	−	Digestion, 5
5	0.3	5 $\frac{n}{6400}$	0.0005	−	Digestion, 3

TABLE C (*continued*).

Period of digestion, 10 minutes. Temperature, 17° C.

No. of Tube.	Diluted saliva added. c.c.	HCl added. c.c.	Total acidity as HCl Per cent.	Reaction for *free* HCl.	Results.		
					Exp. I.	Exp. II.	Exp. III.
1	0.3	$5\,H_2O$	0	−	Digestion, 4	Digestion, 3	Digestion, 4
2	0.3	$5\frac{n}{1000}$	0.0036	+	No digestion	No digestion	No digestion
3	0.3	$5\frac{n}{1500}$	0.0024	−	Digestion, 10	Digestion, 2	Digestion, 2
4	0.3	$5\frac{n}{2000}$	0.0018	−	Digestion, 5	Digestion, 4	Digestion, 5

Second Series. — The following experiments are selected to illustrate how an increase in the amount of neutral saliva added may modify the influence of a definite proportion of acid, presumably owing to a combination of its proteid with the latter. The relatively weaker inhibitory action of combined acid is thus demonstrated.

TABLE D.

Period of digestion, 10 minutes.

No. of Tube.	Diluted saliva added. c.c.	HCl added. c.c.	Total acidity as HCl. Per cent.	Results.	
				Exp. I. Temp. 40° C.	Exp. II. Temp. 17° C.
1	3.3	$2\,H_2O$	0	Digestion, 5	Digestion, 3
2	3.3	$2\frac{n}{1000}$	0.0036	No digestion	No digestion
3	3.3	$2\frac{n}{2000}$	0.0018	Digestion, 12	Digestion, 5

In Experiment I, 3.3 c.c. saliva were added to Tube 2, whereupon the mixture was digested, and gave a color comparable to No. 4 of the color scale. In Experiment II, it required three successive additions of 3.3 saliva to bring about digestion.

TABLE E.

Period of digestion, 10 minutes.   Temperature, 17° C.

No. of Tube.	Diluted saliva added. c.c.	HCl added. c.c.	Total acidity as HCl. Per cent.	Reaction for *free* HCl.	Result.
1	0.3	$5\ H_2O$	0	—	Digestion, 5
2	0.3	$5\frac{n}{1000}$	0.0036	+	No digestion
3	0.3	$5\frac{n}{1500}$	0.0024	?	No digestion
4	0.3	$5\frac{n}{2000}$	0.0018	—	Digestion, 10

At the end of ten minutes 0.3 c.c. saliva was again added to Tubes 2 and 3. Tube 2 still gave an acid reaction, while Tube 3 digested at once, and at the end of ten minutes gave with potassium hydroxide a color reaction slightly weaker than that obtained with Tube 1.

In the following protocols the effect of the reverse process, namely, diminishing the amount of neutral saliva added when the other conditions are unchanged, is illustrated. (See Table F, page 258).

Third Series. — The influence of increased quantities of neutral starch paste in modifying the inhibitory effect of acid is shown below.

TABLE G.

Period of digestion, 10 minutes.   Temperature, 37° C.

No. of Tube.	Starch paste added. c.c.	Water added. c.c.	HCl added. c.c.	Total acidity as HCl. Per cent.	Diluted saliva. c.c.	Result.
1	1	4	$5\frac{n}{1000}$	0.0036	0.3	No digestion
2	2	3	$5\frac{n}{1000}$	0.0036	0.3	No digestion
3	3	2	$5\frac{n}{1000}$	0.0036	0.3	Digestion, 2
4	4	1	$5\frac{n}{1000}$	0.0036	0.3	Digestion, 4
5	5	0	$5\frac{n}{1000}$	0.0036	0.3	Digestion, 5

In Table H the effect of acid of varying strength upon the saliva is shown. It was first ascertained that five c.c. of saliva carefully neutralized to litmus required exactly five c.c. $\frac{1}{10}n$ hydrochloric acid to give the faintest reaction with dimethyl amido-azobenzol.   Equal volumes of saliva were then mixed with varying

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TABLE F.

Period of digestion, 10 minutes. Temperature, 37° C.

No. of Tube.	HCl added. c.c.	Total acidity as HCl. Per cent.	Experiment I.				Experiment II.			
			Diluted saliva. c.c.	Result.	Diluted saliva. c.c.	Result.	Diluted saliva. c.c.	Result.	Diluted saliva. c.c.	Result.
1	$5\,H_2O$	0	1·6	Digestion, 3	3·3	Digestion, 4	0·3	Digestion, 5	0·6	Digestion, 10
2	$5\,\frac{n}{1000}$	0·0036	1·6	No digestion	3·3	Digestion, 3	0·3	No digestion	0·6	Digestion, 1
3	$5\,\frac{n}{1250}$	0·0029	1·6	No digestion	3·3	Digestion, 4	0·3	Digestion, 3	0·6	Digestion, 3
4	$5\,\frac{n}{1500}$	0·0024	1·6	Digestion, 5	3·3	Digestion, 4	—	—	—	—

quantities of hydrochloric acid, the total volume in each case being kept the same. At the end of the intervals indicated, the mixtures were carefully neutralized to litmus, and their digestive power tested with starch paste. The results of two experiments are given in Table II.

TABLE II.

No. of Tube.	Diluted saliva. c.c.	Water added. c.c.	$\frac{N}{100}$ HCl added. c.c.	Total acidity as HCl. Per cent.	Results.	
					Exp I. 30 minutes at 17° C.	Exp. II. 1½ hours at 40° C.
1	5	10	10	0.0146	No digestion	No digestion
2	5	12	8	0.0116	No digestion	No digestion
3	5	13	7	0.0102	Digestion	No digestion
4	5	14	6	0.0081	Digestion	No digestion

From these data it will be seen, as has already been pointed out by Chittenden and Smith, that *free* hydrochloric acid, especially when it reacts for any length of time, is destructive to the enzyme of the saliva, even with extreme dilution of the acid.

With reference to the retarding effect of alkalies upon salivary digestion as ascertained by Kübel, we recall that Chittenden and Smith[1] also pointed out the inhibitory reaction in the case of sodium carbonate. But in this instance they likewise emphasize the importance of considering the dilution of the saliva and consequent changes in the amount of proteid present, before any definite statement can be arrived at for definite strengths of alkali.

In discussing the fate of the saliva in the stomach during digestion, it will hereafter be necessary to take into consideration the investigation of Cannon[2] on the absence of movement in the fundus, if the observations on the cat and dog be applicable to other animals and man also. The food is not readily mixed with the gastric juice in this portion of the organ, and consequently an acid reaction does not develop for some time. Salivary digestion may thus presumably proceed for some time in this region without marked retardation.

[1] CHITTENDEN and SMITH: Studies, etc., p. 33.
[2] CANNON: This journal, 1898, i. p. 379.

SUMMARY.

The chief object of this note has been to point out that it is impossible to designate any percentage of acid or alkali which inhibits salivary digestion in a definite degree. The character of the action is dependent also upon the absolute amount of saliva and the attendant variation in the quantity of proteid matter present. Whenever *free* hydrochloric acid is present, inhibition — more or less complete — is certain to result.

IV.

ON THE CONNECTIVE TISSUE IN MUSCLE.

BY J. H. GOODMAN.

IN a paper from the hygienic institute in Würzburg, E. Schepilewsky [1] has quite recently described a new method for the determination of connective tissue in muscle. Strips of meat are gently rubbed in a mortar with water, the latter being repeatedly renewed. It is possible to remove the bulk of the true muscular tissue in this mechanical way, and to retain the meshwork of connective tissue. The water is next poured through a fine sieve which holds the detached pieces of connective tissue, but allows the muscle elements to pass through. According to Schepilewsky, practically all of the connective tissue can thus be retained, while only small quantities of muscle fibres themselves are held back in the meshwork of tissue. The latter is now rubbed up with five per cent sodium hydroxide solution and allowed to stand in contact with the alkali for some time. The true muscle elements are found entirely dissolved at the end of fifteen hours; the connective tissue swells up and becomes transparent, showing the elastic fibres in clear outline. The undissolved mass is thereupon filtered through a perforated porcelain plate covered with cotton-wool and is washed well with water; the entire residue, including cotton, is next boiled gently for five or ten minutes with a small volume of half per cent sodium hydroxide solution. The collagen passes into solution in the hot alkali, leaving the elastic fibres undissolved. By determining the nitrogen in a portion of the filtered

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[1] SCHEPILEWSKY: Archiv für Hygiene, 1899, xxxiv, p. 348.

solution, the amount of collagen dissolved can readily be estimated. In discussing the details of the method, Schepilewsky writes as follows, regarding the action of the strong alkali: "The lye dissolves the proteids, at the same time saponifying the fats and extracting from the connective tissue the greater part of the *mucin;* the latter is readily detected by adding an excess of acetic acid to the filtered alkaline fluid. The proteids present in the latter are redissolved by this procedure, while the *mucin* is precipitated in flocks." Again, in reference to the final alkaline fluid obtained after the treatment with the more dilute alkali, it is stated: "There may still be present a small quantity of *mucin*, which is readily removed by adding acetic acid in excess and filtering off the precipitate formed after some time; fatty acids are also retained on the filter." [1]

Although we have been unable to find any reference to the occurrence of mucin in muscles, it did not seem improbable that this compound proteid might be present; for mucin is a characteristic component of white fibrous connective tissue, and apparently also of bone. [2] We have therefore separated the connective tissue from muscle and isolated the so-called mucin described by Schepilewsky. Three different samples of muscular tissue were examined, two of which were lean beef, the third being rabbit's muscle. In each instance about five hundred grams of the meat were chopped up, rubbed in a mortar repeatedly with water and strained, until practically all of the muscle fibres were eliminated. The connective tissue residue was then placed in five per cent sodium hydroxide solution for about fifteen hours. The resulting extract was strained off and filtered; the filtrate was acidified with acetic acid and the flocculent precipitate obtained was washed thoroughly with water by decantation, with alcohol and ether, and dried at 105° C. This was preparation A, which might be made up in part or entirely of mucin, as the latter is readily soluble in alkalies, but insoluble in excess of acid. The residue undissolved after treatment with the caustic alkali was treated with half per cent sodium hydroxide solution, in which the collagenous tissue dissolved together with some saponified fats. The solution was filtered and acidified with an excess of acetic acid, which threw down a precipitate of proteid and fatty acids. This precipitate would not dissolve completely in

[1] Schepilewsky: *loc. cit.*, pp. 357–358.
[2] Gies: This journal, 1900, iii, p. vii.

a very large excess of acid; on treatment with boiling alcohol, nearly all of it went into solution as fatty acid, leaving only a trace of proteid behind. Portions of fatty acid obtained in this way melted above 62° C.

In order to determine the identity of preparation A as mucin, portions of about 1.5 grams were heated for fourteen hours with two per cent hydrochloric acid. It was hoped to bring about a cleavage of the glycoproteid in this way, if mucin were present.[1] The solution was nearly neutralized (never becoming alkaline), and was concentrated to a small volume. The presence of a soluble carbohydrate was tested for with Fehling's solution. No reduction was ever observed, and *no carbohydrate group could be detected in any preparation of the so-called mucin.* Furthermore, the mucins are all characterized by a relatively low content of nitrogen in contrast with simple proteids. Thus tendon mucin contains less than 12 per cent of N, while snail mucin and submaxillary mucin contain 13.6 and 12.3 per cent respectively.[2] Analyses of the preparations from muscle gave far higher results, as will be seen in the summary below. The nitrogen determinations were made in duplicate by the Kjeldahl-Gunning method. The figures given are calculated for the ash-free substance. Since the nucleoproteids resemble the true mucins in their solubilities, phosphorus determinations were made by Hammarsten's method, in order to ascertain whether the material under investigation belonged to the former class. Only traces of phosphorus — less than 0.01 per cent — were found, and these were evidently due to adherent phosphate also detected in the ash.

ANALYSES OF PREPARATION A.

Source.	Ash. Per cent.	Nitrogen. Per cent.	Carbohydrate.
1. Beef	0.66	16.07	None
2. "	0.84	16.22	None
3. Rabbit	1.20	16.02	None

From the preceding considerations it is evident that the material assumed by Schepilewsky to be mucin is neither a glycoproteid nor

[1] CHITTENDEN and GIES : Journal of experimental medicine, 1896, i, p. 186.
[2] HALLIBURTON : Schaefer's textbook of physiology, 1898, i, p. 62.

a nucleoproteid. Its composition and solubilities recall the *stroma substance* of J. von Holmgren.[1] This investigator, repeating Danilewsky's experiments,[2] treated the insoluble muscle stroma remaining after complete extraction of muscle with water and ammonium chloride solution, with dilute alkali. He obtained in this way from horse and rabbit muscle a proteid precipitable by acids and yielding neither xanthin bases nor reducing substances. Von Holmgren's stroma proteid contained from 15.84 to 16.66 per cent of nitrogen.

In speaking of the final filtrate — the gelatin solution obtained by dissolving the connective tissue in hot alkali — Schepilewsky says: "It gives no coloration with Millon's reagent, if the proteids have actually been removed."[3] It has been taught quite universally that pure gelatin does not give Millon's reaction; the latter, when obtained with gelatin solutions, is attributed to contaminating proteids.[4] But the investigations of Van Name[5] in this laboratory have demonstrated that perfectly pure gelatin, prepared from connective tissue, still gives a red coloration when warmed with Millon's reagent; and this observation has received confirmation in the recent work of C. Th. Mörner.[6] In order to test the matter still further we have neutralized and concentrated the gelatin-containing filtrates obtained by Schepilewsky's method, and have precipitated the albuminoid material with alcohol. The precipitate of gelatin (or gelatoses) gave the characteristic red coloration with Millon's reagent.

[1] Von Holmgren : Jahresbericht für Thierchemie, 1893, xxiii, p. 360.

[2] Danilewsky : Zeitschrift für physiologische Chemie, 1882, vii, p. 124.

[3] Schepilewsky : *loc. cit.*, p. 358.

[4] *E.g.* Neumeister : Lehrbuch der physiologischen Chemie, 1897, p. 63. Salkowski : Practicum der physiologischen und pathologischen Chemie, 1900, p. 199.

[5] Van Name : Journal of experimental medicine, 1897, ii, p. 117.

[6] C. Th. Mörner : Zeitschrift für physiologische Chemie, 1899, xxviii, p. 484.
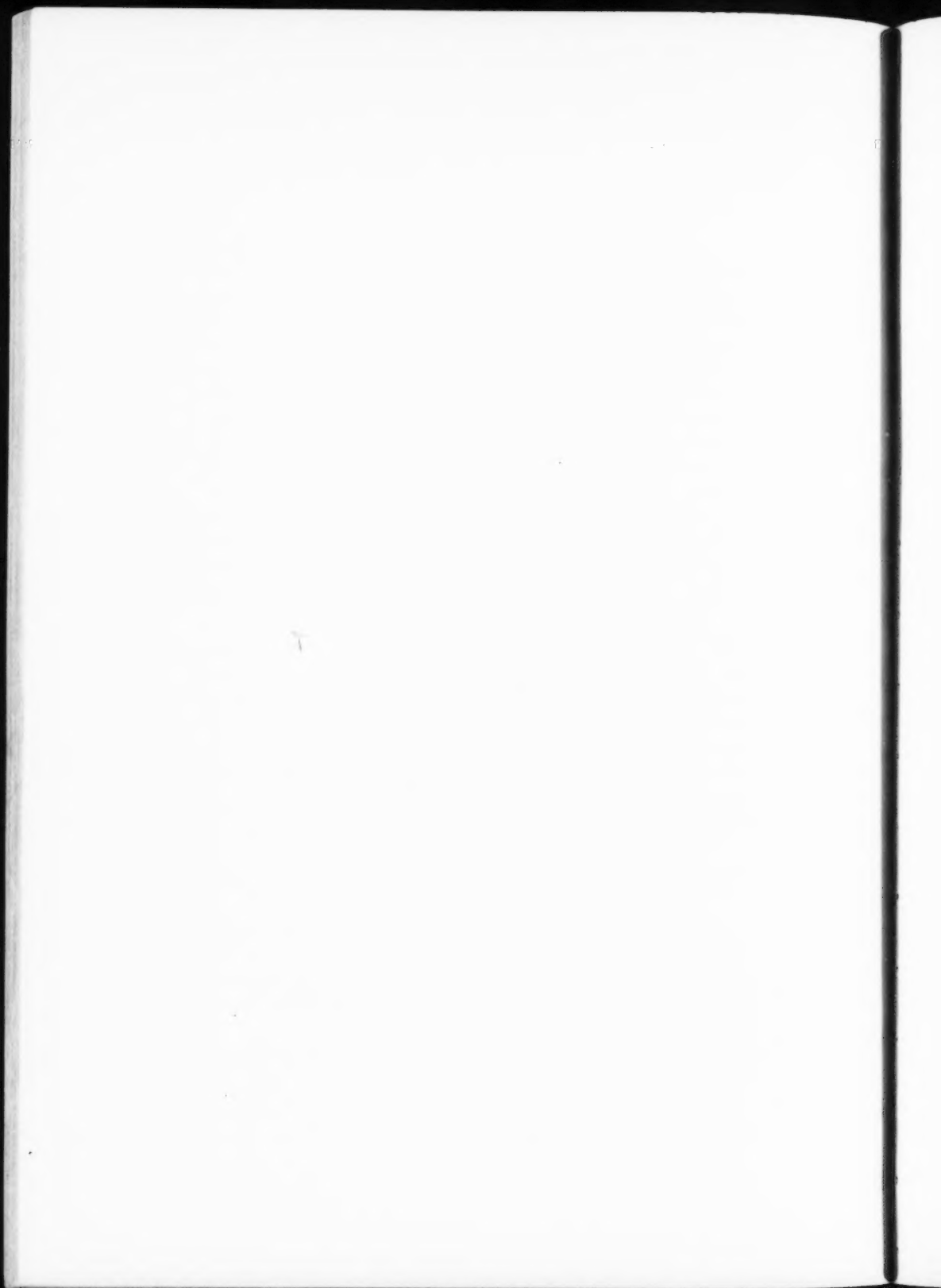
THE ACTION OF CERTAIN IONS ON VENTRICULAR MUSCLE.

By D. J. LINGLE.

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CONTENTS.

I. INTRODUCTION.

GAULE, Ringer, Howell, and others, maintain that the origin of the heart's rhythmic activity must be sought among the inorganic constituents of the blood. The latest theory to explain this action has been advanced by Howell,[1] who holds that to produce rhythmic contractions in heart tissue the interaction of three salts is necessary — sodium chloride to maintain osmotic pressure, calcium salts to produce tone and act as the real excitant, and potassium salts to cause relaxation and neutralize the excessive stimulating action of the calcium salts. The idea of the rhythmic activity of the heart being a function of the inorganic salts of the blood is indirectly supported by some work by Loeb in another field. He has shown that rhythmic contractions can be produced at will in striped muscle of the frog by the action of a single salt solution, thus contradicting Biedermann's supposition that a mixture of salts is necessary. This effect he believes is produced not by the salt itself but by ions, because it occurs only in solutions of electrolytes — i. e. substances that dissociate. Among the ions found in blood he thinks those of sodium are the producers of rhythmic activity. They constitute the primary stimulus. Hence a pure solution of sodium chloride is not

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[1] HOWELL: This journal, 1898, ii, p. 47.

a neutral medium as has been supposed for years, but is physiologically active. A pure sodium chloride solution may be regarded as a poison.[1] If rhythmic activity begun by it is to persist, these poisonous properties must be neutralized by calcium salts. Loeb thinks calcium and potassium salts prevent rhythmic activity, but that they in conjunction with sodium chloride bring about a sustained rhythm. Loeb and Howell thus agree in that each considers the interaction of sodium chloride, calcium salts, and potassium salts necessary for the production of a sustained rhythm. Loeb's conclusions were reached by a study of the action of salt solutions on frog muscle and on the rhythmic tissue in the umbrella of Gonionemus.

In this paper an attempt has been made to see whether Loeb's ideas hold true for ventricular tissue of the turtle. Special attention has been given to the following points : —

1. Will ventricular tissue beat rhythmically in solutions of the nonconductors, or, in other words, is rhythmic activity an ion effect in heart muscle?

2. If the stimulus for this rhythmic activity is an ion, is it the calcium or sodium ion?

3. What is the rôle of other ions with respect to ventricular rhythmic activity?

II. METHODS.

The preparations experimented upon consisted of slender strips cut from the ventricle of the turtle, Chrysemys marginata. In making them the ventricle was cut through a little below the auriculo-ventricular groove. A second cut parallel and two or three millimetres below the first removed from the ventricle a complete ring of tissue. The ring was next cut at opposite sides so as to form two pieces as near alike physically and anatomically as possible. Each piece was usually about ten millimetres long by two or three millimetres thick. In many experiments both strips were used at the same time with one as a control. The heart was never washed out with physiological salt solution before making the strips because such treatment so modifies the tissue that it will not react to salt solutions in the manner of normal muscle. The cut slips were placed on filter paper and as much blood removed as possible. In some cases the innermost more spongy layer of tissue was shaved off

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[1] LOEB: This journal, 1900, iii, p. 327.

and more blood thus removed, but since this procedure did not seem to modify the results it was not always carried out. One end of each strip was fastened to a light lever by a silk thread, the other was tied to the lower end of a stationary L-shaped glass rod, directly below the lever. This rod with the muscle could be submerged in a beaker containing the test solution, and the contractions of the strip recorded on a slowly moving drum. Usually 50 or 100 c.c. of solution were used at a time. When a strip was transferred from one solution to another the beaker with its contents was removed and a new one substituted. In this way there was the least possible mixing of successive liquids.

It is important to note that the strips of heart tissue used had in them a certain amount of blood, and also that they were quiescent as a result of operative violence. They were cut, however, from beating ventricles, and all of the following experiments deal with strips which were in this condition at the beginning of the experiment. Later, of course, experiments were made on beating strips and strips stopped by other means than operative violence.

III. Action of Solutions of Nonconductors.

The first point was to determine whether strips of heart ventricle prepared as described would originate and maintain rhythmic beats in solutions of nonconductors. To test this a series of experiments was made with solutions of cane sugar, dextrose, and glycerine.

Cane Sugar. — It was found that strips of ventricle when placed in a solution of cane sugar equimolecular with a 0.7 per cent sodium chloride solution did not beat rhythmically. In one case a strip made two beats at a considerable interval apart, but there was no rhythm and usually the strips in cane sugar remained absolutely quiet until dead. This failure to beat was not caused by the sugar solution killing the strips before they had time to begin, for after a long immersion rhythmic activity would begin when the proper electrolyte was added.

Dextrose. — Greene noticed that a strip of ventricle placed in an isotonic solution of this sugar was thrown into a condition of tone and gave a series of somewhat irregular contractions which lasted a variable, but always a short time.[1] In this instance a rhythm was

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[1] GREENE: This journal, 1898, ii. p. 126.

established in a solution of a nonconductor, a fact that directly con-
tradicts Loeb's idea. I repeated Greene's experiment, using a solu-
tion of dextrose equimolecular with a 0.7 per cent sodium chloride
solution, and got rhythmic contractions in the form of an irregular
rhythm lasting about an hour. In this preparation there was an evi-
dent tendency for the beats to appear in groups of two to eight beats
with short pauses between the groups. (See Fig. 1.) The dextrose
solution used was made from the ordinary so-called granular chemi-
cally pure dextrose. When it was tested by the flame test the color
indicated the presence of a considerable amount of sodium. The
color was much more marked than with cane sugar of the same
strength. Dr. Stieglitz kindly analyzed the solution for me and
found it contained considerable sodium chloride. Through the

FIGURE 1. — Illustrates the nature and extent of a rhythm established in a solution of
so-called chemically pure dextrose. This and subsequent tracings should be read
from left to right.

kindness of Professor Nef I secured a sample of *crystalline* dextrose.
A solution of this was as clear as distilled water and showed when
tested no trace of sodium chloride. *Strips of ventricle when placed
in it did not beat rhythmically nor would they make even single con-
tractions.* The only change was an increase in tone of the strip. It
is possible that those who have obtained rhythmic beats from strips
in dextrose solutions did not use the crystalline form of the sugar,
and so really worked with a dilute solution of sodium chloride in
dextrose. The crystalline dextrose solution, like the cane sugar
solution, does not kill the heart tissue for some time, and strips that
will not beat in it can be made to do so by putting them into a solu-
tion of sodium salts.

Glycerine. — When strips of ventricle were placed in a solution of
glycerine equimolecular with 0.7 per cent sodium chloride no con-
tractions occurred. There was a slight increase in tone, but this was

not so great as with the dextrose solution. The glycerine solution seems to be more injurious to the tissue than either dextrose or cane sugar; for the strips in it die sooner, and it is more difficult to get them to contract rhythmically after being under its influence.

In these three classes of experiments with nonconductors an opportunity is given of testing one side of Howell's theory regarding the action of salt solutions causing rhythmic contractions in heart muscle.[1] He explains the fact that nonbeating heart-strips when placed in a pure sodium chloride solution beat rhythmically, as follows. In such strips calcium and potassium constituents are present in proportions that neutralize the stimulating effect of the calcium compounds. When, however, the ventricular tissue is placed in a solution of sodium chloride that is isotonic to the blood, a diffusion of the calcium and potassium constituents may be supposed to take place from the liquids of the tissue to the bath of sodium chloride. If the rapidity of diffusion of the calcium constituent is less than that of the potassium constituent "the normal balance between them is disturbed, and a preponderance of the calcium compounds results sufficient to stimulate the muscle to contraction." In the experiments just described the solutions of cane sugar, dextrose, and glycerine were equimolecular with a 0.7 per cent sodium chloride solution, and the diffusion Howell postulates of calcium and potassium compounds should have taken place. A preponderance of calcium compounds should have resulted and rhythmic beats followed. The fact that no rhythm was developed in these solutions supports Loeb's conclusions that rhythmic activity occurs only in solutions of electrolytes and is therefore an ion effect, and is against Howell's theory that the interaction of calcium and potassium salts causes rhythmic contractions. The bearing of these experiments on this point will be referred to again.

If rhythmic contractions are an ion effect, the question arises what ion is most closely related to the origination of rhythmic beats in heart-tissue? Ringer and Howell assert that rhythmic heart-beats are the result of the interaction in its tissue of calcium and potassium compounds. In this reaction calcium compounds are the actual stimulus to contraction, while the potassium compounds produce elongation and neutralize the calcium. Loeb maintains that the stimulus is most closely associated with the sodium compounds or

[1] HOWELL: *loc. cit.*, p. 79.

rather with the sodium ion. The calcium and potassium salts simply prolong the rhythm and improve it by their ability to neutralize the injurious effects of too much or too extended action of sodium. There is a definite issue here, and the question to settle is this: Do the sodium salts or the calcium salts furnish the stimulus for the heart-beat? The best way to answer this question is to study separately and together the action on heart-tissue of the three ions, sodium, calcium, and potassium. It is important to distinguish at the outset between agents that *start* and those that *maintain* rhythmic beats in heart-tissue; they may not be identical. All solutions used in these experiments were equimolecular with a 0.7 per cent sodium chloride solution unless otherwise stated.

IV. Action of Sodium Ions.

When strips of nonbeating ventricle are placed in a 0.7 per cent solution of sodium chloride they always beat rhythmically. In a large number of experiments during a whole year not one failure to start beats by this solution was recorded. In this rhythm there is a latent period lasting from a few minutes to over an hour. The beats may begin with considerable strength or sometimes may develop to a maximum gradually. If the strip is kept in the solution the beats reach a maximum and then gradually decline to a complete standstill. The whole series of contractions lasts as a rule from an hour to three hours. Howell and Greene both noticed this rhythmic action of heart-strips in sodium chloride. Greene [1] has published a tracing showing the phenomena and Howell [2] states that heart-strips will always beat rhythmically in physiological salt solution. But Howell does not think the sodium solution starts these beats. He attributes them to the action of calcium and potassium compounds in the tissue. These compounds, as has been stated, he thinks diffuse out with unequal speed, and so get into such proportions that calcium can exert a stimulating effect. The facts given in considering the effects of nonconductors on heart-tissue are against this idea, and still other facts can be presented. When a strip of ventricle is placed in a solution of cane sugar equimolecular with 0.7 per cent sodium chloride no beats occur in the strip. But when the strip is transferred from the sugar solution to a 0.7 per cent sodium chloride solution rhythmic beats sometimes begin suddenly, so suddenly that

[1] Greene: *loc. cit.*, p. 105.   [2] Howell: *loc. cit.*, p. 71.

they seem as if started by an electric shock. (See Fig. 2.) Again it can be shown that to some extent the power a solution has of starting rhythmic beats in heart-tissue is determined by the number of sodium ions present in it. For example, a solution consisting of 49 c.c. of cane sugar + 1 c.c. of sodium chloride did not start beats. Nor would a strip beat in a solution of 40 c.c. sugar + 10 c.c. of sodium chloride, but this strip did beat when it was transferred to a solution of 25 c.c. of sugar + 25 c.c. of sodium chloride solution. The same results were obtained with a solution of dextrose. We can
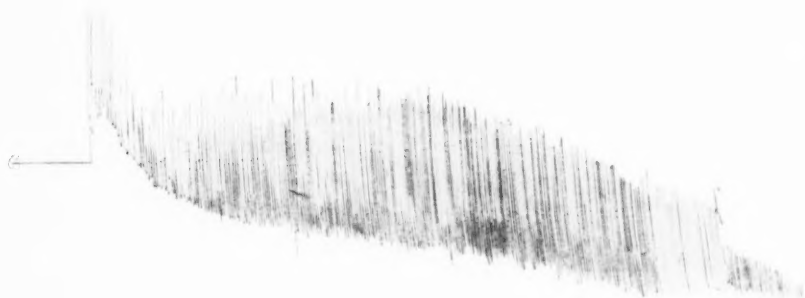
FIGURE 2. — A strip of ventricle put into a solution of cane sugar at 10.20 A.M. did not contract. At 11.35 it was transferred to a 0.7 per cent sodium chloride solution, when beats began instantly.

then have solutions of sodium chloride in sugar that will not start beats and others that will. The effective solutions are those with the greater percentage of sodium ions in them. This fact can be demonstrated in another way. A solution of lithium chloride equimolecular with 0.7 per cent sodium chloride has no power to start rhythmic contractions in isolated strips of ventricle tissue. But such a solution with sodium chloride added will start and maintain rhythmic beats for some time. That the power of the mixture to cause contractions is to some extent conditioned by the percentage of sodium chloride in the solution, the following table shows : —

TABLE I.

			Remarks.
Solution 1.	2 c.c. NaCl + 48 c.c. LiCl.	No beats.
" 2.	5 c.c. NaCl + 45 c.c. LiCl.	Strong beats with a slow and somewhat imperfect rhythm, which did not last long.
" 3.	10 c.c. NaCl + 40 c.c. LiCl.	Good strong beats lasting longer than before, and with a faster rhythm.
" 4.	15 c.c. NaCl + 35 c.c. LiCl.	Good strong beats; rhythm still slower than control in pure NaCl.
" 5.	20 c.c. NaCl + 30 c.c. LiCl.	Same as above.
" 6.	25 c.c. NaCl + 25 c.c. LiCl.	Beats strong with gradual cessation in 3 hours. Not so rapid a rhythm as in pure NaCl.

Of course the strips used came from a number of hearts, and it is not possible to compare them closely. But it seems that the first solution did not have a sufficient number of sodium ions to start beats. As the amount of sodium was increased the latent period shortened. For example, two strips from the same heart were placed, one in 25 c.c. NaCl + 25 c.c. LiCl and the other in 35 c.c. LiCl + 15 c.c. NaCl. The latent period of the first was 42 minutes shorter than that of the latter. The rate was not so rapid in the dilute solutions of sodium as in the control with pure sodium chloride.

If sodium chloride is so closely associated with the real stimulus, the sodium and chlorine ions of which it is composed must now be considered. Loeb working on striped muscle and Gonionemus tissue found that the sodium ion was the active agent. In heart-tissue the same seems to be true, for solutions containing sodium, but no chlorine, are able to do the work of sodium chloride, and, indeed can do its work even better. Among such solutions is sodium bromide. A NaBr solution equimolecular with a 0.7 per cent NaCl solution, if properly used, will start rhythmic beats in heart-strips; the beats so started are generally stronger than those in sodium chloride, and moreover the rhythm lasts longer.

Other facts will be given later that perhaps more strongly indicate that it is the sodium ion that furnishes the actual stimulus for the beats in heart-muscle. Loeb thinks the sodium ion acts by migrating into the muscle substance and combining with some part of it. And hence when too many sodium ions have combined and taken the place of a number of calcium ions in the muscle rhythmic beats cease. Muscle in this condition, termed by Greene the "sodium chloride standstill," is most interesting physiologically from the

standpoint of the theory that the heart-beat is caused by inorganic salts. Some attempts were made to test indirectly on heart-muscle Loeb's hypothesis. First the rapidity with which the sodium ions take the place of the other ions in the heart-tissue was slowed by diminishing the number of ions in the unit of solution. According to the theory this diminution should cause a slower and more prolonged rhythm. To test this two strips from the same heart were compared. One (Fig. 3, *a*) was put into a solution of 30 c.c. of lithium chloride + 20 c.c. of sodium chloride. The other (*b*) was treated with pure sodium chloride solution. The first strip in a solution with fewer sodium ions beat both slower and longer than the latter; the rate cannot be given accurately but the duration of the rhythmic activity was very nearly three times as long as that of the second strip. A like result was secured by another method. Similar strips were taken from the same heart. One was permitted to start beats in a pure 0.7

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FIGURE 3.—Tracing *a* was made by a strip of heart muscle in a solution of 30 c.c. LiCl + 20 c.c. NaCl, while *b* was made by a strip from the same heart in a pure NaCl solution. The latent period in *a* was 30 minutes; in *b*, 16 minutes. The rate is slower in *a*, the duration of the rhythm greater, and the individual beats stronger.

FIGURE 4. — At 10.33 A.M. a strip was put into a solution of 0.7 per cent NaCl, and permitted nearly to run down (11.15 A.M.). It was then transferred to a solution of cane sugar, equimolecular with 0.7 per cent NaCl, which caused an increase in force of the beats, but a slower rhythm. The moment of transfer is indicated by the arrow.

per cent sodium chloride solution and was left there until the beats ceased. The second strip was placed in the same solution, and, shortly after the rhythm began, before the force of the beats was maximal, was removed from the solution and placed in a moist chamber. The rhythm of the first strip lasted one hour and thirty minutes; that of the second more than eight hours. In fact, the latter seemed to stop because the moist chamber was imperfect and permitted the strip to dry during the night. In this case a surplus of sodium ions was impossible and their poisonous effects were thus avoided. The remarkable fact here is that a short application of sodium ions (three minutes) kept the strip in activity for so long a time. The sodium must have acted simply to start the contractions. This experiment may explain the fact that strips of ventricle will sometimes beat rhythmically when suspended by the method devised by Gaskell. In such experiments it has been the custom to moisten the

preparation with physiological salt solution, that is, to give the strip enough sodium ions to start it contracting. It is important to know if rhythmic beats are possible without the application of a sodium salt solution. Now as to the other side of the theory. If an excess of sodium ions is injurious, how will a strip suffering from an excess be affected by the removal of some of the ions? The removal should modify the rhythm; and so it does, — it improves the force of the beats, but, as would be expected, slows the rate. This effect is shown in Fig. 4. The slowing continues to a complete standstill. The arrest is clearly due to a lack of sodium ions, for a rapid recovery takes place when the sodium ions are restored to the strip. (See Fig. 5.)

If we can draw any conclusions from these experiments with sodium chloride it seems to me they support Loeb's ideas. We must therefore believe that the same ion that starts rhythmic contractions in the striped muscle of the frog and the central portion of the bell of Gonionemus also starts those of heart-tissue. Howell[1] noticed similar facts; he states, for example, that strips of ventricle apex that will not beat when suspended by Gaskell's method do so when placed in a sodium chloride solution. Again[2] he states that strips placed in serum do not beat, but begin to beat when placed in physiological salt solution. Greene[3] notes that strips which do not beat in fresh serum begin to beat when placed in sodium chloride solution of 0.6 per cent, and he

[1] HOWELL: *loc. cit.*, p. 71.
[2] HOWELL: *loc. cit.*, p. 72.
[3] GREENE: *loc. cit.*, p. 88.

FIGURE 5. — A strip which began to beat in NaCl solution was placed at 11.51 in cane sugar solution, which caused a slower rhythm. When the strip was again placed in NaCl solution at 12.37 the rhythm reappeared.

gives [1] a table showing the latent period, height of contraction, and rate of rhythm of strips in sodium chloride solutions of 0.6 to 0.7 per cent. It seems to me the simplest possible interpretation of these facts is that sodium chloride is the actual stimulus, and not calcium as Howell declares.

V. Action of Calcium Ions.

Greene [2] states that a solution of calcium chloride in approximately isotonic solutions, when applied to a heart-strip, throws the muscle into strong tone, but will not cause contractions during five minutes' immersion. When, however, the excess of calcium is removed by washing with 0.7 per cent sodium chloride solution he says that a series of very rapid contractions immediately starts up. Is not this a sodium chloride effect? Greene also found that the application of a solution of calcium chloride of the percentage found in blood would cause contractions. The use of such a solution introduces physical changes which must play a rôle that we do not understand. At any rate the action of such a solution cannot be considered a simple calcium effect. I find that a strong solution of calcium chloride equimolecular with 0.7 per cent sodium chloride does not start contractions in heart-strips. It can, however, stop contractions in strips that are active. According to Howell's theory, if calcium is to start rhythmic beats, its proportions in the nonbeating tissue must be changed or the proportions of the neutralizing potassium compounds must be varied. It has been shown that when conditions are such that potassium can diffuse from the tissue more rapidly than calcium, *i. e.* in sugar solution, no beats occur. This point may also be tested by observing whether the addition of calcium to the tissue overcomes the inhibitory effect of the potassium, and starts the beats. Here the problem is one of proportion. A strip was placed in a solution consisting of 47 c.c. lithium chloride + 3 c.c. calcium chloride. Both solutions were equimolecular with 0.7 per cent sodium chloride. The strip beat twice in one hour and forty minutes. It was then transferred to a solution of 47 c.c. of sodium chloride + 3 c.c. of calcium chloride upon which beats began in 8 minutes and continued with a perfect rhythm. As a control the other strip from this heart was put into 47 c.c. of sodium

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[1] Greene: *loc. cit.*, p. 92.　　　[2] Greene: *loc. cit.*, p. 101.

chloride + 3 c.c. of calcium chloride. Beats appeared in this piece thirteen minutes after immersion. In these experiments it is clear that solutions of calcium compounds failed to start a rhythm while solutions containing sodium succeeded. Calcium, though unable to start rhythmic beats, could stop them, for when the beating strip was transferred from the solution containing sodium to one consisting of 47 c.c. lithium chloride + 3 c.c. calcium chloride they ceased. It may be objected that the proportion of calcium was too large in these experiments. But the result was the same with solutions of 49 or 49.5 c.c. lithium chloride and 1 or 0.5 c.c. of calcium solution, and these proportions can hardly be too great.

So far calcium has been treated in two ways. By the first method in combination with sugar solution. The conditions favored an adjustment of the proportions of calcium and potassium in the tissue of such kind that calcium predominated. By the more rapid diffusion of potassium the calcium percentage became greater. Here, if calcium had stimulating powers, they should have been active, but they did not appear. With the second method a positive increase in the amount of calcium in the tissue was produced. Moreover, while the calcium proportion was increased a slow diffusion of potassium out of the tissue went on, which made the proportions of calcium and potassium vary more markedly. Under these conditions calcium again failed to start rhythmic beats in strips of heart-ventricle.

It is certain then that under the conditions described calcium ions cannot start rhythmic beats when sodium ions do start them. In fact I could not get calcium to start beats under any conditions. If these results are correct calcium cannot be the stimulus for the rhythmic contractions of heart-muscle.

Calcium, however, certainly plays an important part in the rhythmic activity of the heart and other tissues as a sustainer of the activity. When strips of heart-ventricle beating in sodium chloride are given the proper amount of calcium chloride the force of the individual beats is increased and the duration of the rhythm lengthened. And this favorable action is exerted without the presence of potassium compounds. I attempted to determine the proportion of calcium salt most favorable for sustaining the rhythm in isolated heart-strips, using for the purpose a solution of calcium chloride equimolecular with a 0.7 per cent sodium chloride solution. Since the results were obtained from a study of a number of strips from different hearts, allowance must be made for individual differences

in tissue. I think the results numerous enough, however, to permit a rough generalization to be made.

TABLE II.

Solution 1.	49.5 c.c. NaCl + 0.5 c.c. CaCl₂.		Beats stronger and rhythm longer than in NaCl solution.

Solution   1.   49.5 c.c. NaCl + 0.5 c.c. $CaCl_2$.   Beats stronger and rhythm longer than in NaCl solution.

"   2.   49   c.c. NaCl + 1   c.c. $CaCl_2$.
"   3.   48.5 c.c. NaCl + 1.5 c.c. $CaCl_2$.   Rhythm was best in these solutions, was
"   4.   48   c.c. NaCl + 2   c.c. $CaCl_2$.   most regular, and lasted longest with
"   5.   47.5 c.c. NaCl + 2.5 c.c. $CaCl_2$.   good force in the beats.
"   6.   47   c.c. NaCl + 3   c.c. $CaCl_2$.

"   7.   46   c.c. NaCl + 4   c.c. $CaCl_2$.
"   8.   45   c.c. NaCl + 5   c.c. $CaCl_2$.   Rhythm nearly the same as in NaCl
"   9.   44   c.c. NaCl + 6   c.c. $CaCl_2$.   solution.
"   10.   43   c.c. NaCl + 7   c.c. $CaCl_2$.

"   11.   42   c.c. NaCl + 8   c.c. $CaCl_2$.   Rhythm not so good as in NaCl solution.
"   12.   41   c.c. NaCl + 9   c.c. $CaCl_2$.

11.52   11.54   11.58   12.05
$CaCl_2$ Na_2SO_4 $CaCl_2$ $NaSO_4$
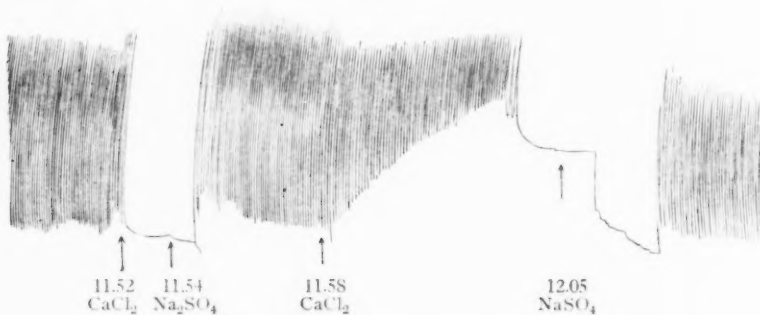
FIGURE 6. — Shows how a solution of $CaCl_2$, equimolecular with 0.7 per cent NaCl will stop rhythmic activity. The tracing also shows how activity thus stopped can be restored by precipitating the calcium with a solution of Na_2SO_4 equimolecular with 0.7 per cent NaCl.

The action of calcium presents another point of interest. Calcium has the power to stop the action of a beating strip. This standstill can be removed by transferring the strip to a solution of pure sodium chloride or by precipitating the excess of calcium with sodium sulphate. In Fig. 6 is shown the action of sodium sulphate. A powerfully beating strip was stopped at 11.52 by the action of calcium chloride. At 11.54 it was treated with sodium sulphate, in solution equimolecular with 0.7 per cent sodium chloride, and at 11.55 beats reappeared. The result can be secured a number of times in succession. This action is analogous to that of atropin and

muscarin, and may some time throw light on the puzzling effect of these alkaloids. Calcium, then, has power to stop rhythmic activity. I tried to see if it plays a rôle in the standstill produced in ventricular strips by cutting. When such strips are put at once into the sodium sulphate solution they will not beat. Clearly if my proportions were correct calcium is not responsible for the nonactivity of such strips.

VI. Action of Potassium Ions.

A solution of potassium chloride equimolecular with 0.7 per cent sodium chloride will not start rhythmic contractions in strips of ventricular tissue. The potassium ions seem to influence the rhythmic activity of tissues by modifying the effects of sodium chloride. Greene [1] thinks they also affect the tissue and produce elongation. He states that heart-strips in an isotonic solution of sodium chloride + 0.03 per cent of potassium chloride always show a great loss of tone. With the proportions of potassium chloride I used I could not get this result. A solution equimolecular with 0.7 per cent sodium chloride always causes a tonic shortening in heart-strips that are not beating. And with two beating strips from the same heart, one in 48 c.c. sodium chloride + 2 c.c. of this potassium chloride solution, and the other in pure sodium chloride solution, the elongation or loss of tone in one was no greater than in the other. It may be that the amount of potassium was too large here, but a solution with 1 c.c. of potassium chloride gave a similar result. These results agree with some unpublished observations of Loeb on the muscle of the frog. As Greene has stated, when beating strips are placed in weak solutions of potassium chloride the rhythm is slowed, the force of the beats weakened and the latent period lengthened. It has been said that the rôle of potassium is to assist in neutralizing the injurious effects of sodium chloride. This idea is supported by an experiment on a strip that had just ceased beating in sodium chloride, and was immediately transferred to a solution of sodium chloride + 1 c.c. potassium chloride. Under these conditions there was established a new rhythm which lasted about an hour. The strongest beat in this rhythm, however, was not one-twentieth that of the maximum in sodium chloride.

[1] Greene: *loc. cit.*, p. 103.

VII. Combined Action of Sodium, Potassium, and Calcium Ions.

So far the action of the various salts or rather ions have been considered separately with the idea of differentiating their effects, if possible. All theories as to the action of inorganic salts on heart-tissue agree that the cardiac rhythm is a result of the interaction of at least three salts, namely the salts of sodium, potassium and calcium. When the combined effect of these salts on cardiac tissue is studied, the result strongly supports the fundamental idea of this paper, that the sodium ion, and not the salts of calcium, is the active agent in producing rhythmic activity in heart-muscle.

According to Howell, sodium chloride maintains the osmotic pres-


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        11.27          11.35                              11.49
     49 c.c. sugar +   added                              NaCl
     1 c.c. CaCl₂      1 c.c. KCl
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FIGURE 7. — At 10.37 a strip of heart muscle was placed in a solution of cane sugar, but it failed to beat; it was transferred to 49 c.c. sugar + 1 c.c. CaCl₂ solution at 11.27, and at 11.35 1 c.c. KCl solution was added without beats appearing. At 11.49 it was put into NaCl solution, and 30 seconds later beats appeared.

sure of the tissue. With this condition attained and proper amounts of calcium and potassium chlorides added, rhythmic contractions should take place. Some experiments were made with different proportions of calcium and potassium chlorides in combination with solutions of cane sugar, glycerine, dextrose, and sodium chloride.

Quiet strips of ventricle were put into a solution of 49 c.c. cane sugar + 1 c.c. calcium chloride + 1 c.c. potassium chloride and failed to contract. But when a strip that had failed to contract was transferred to a solution of sodium chloride, good rhythmic beats began in thirty seconds, and continued for some time. (See Fig. 7.) In this experiment it may be urged that the failure with the mixed salts was due to the incorrect proportions of calcium and potassium. But if so, the failure is due to the small amounts employed, for the salt solutions were added a drop at a time, and carefully mixed by stirring. Stronger mixtures, however, worked no better. A solution of 46 c.c. cane sugar + 2 c.c. of calcium chloride + 2 c.c. of potassium chloride was equally unsuccessful. But when these strips

were transferred to a solution of sodium chloride they beat instantly. The experiment was varied by adding a drop at a time 0.5 c.c. calcium chloride to a solution of 50 c.c. cane sugar, and then adding 0.1 c.c. potassium chloride. As this solution did not start beats, 0.4 c.c. more of potassium chloride was added, which produced a great increase in tone but no beats. These strips, like all others, beat when put into a sodium chloride solution. Greene [1] gives a summary of the percentage of salts most favorable for the development of rhythmic beats in heart-tissue. I have used these proportions to make certain that the failure to get rhythmic contractions was not due to my neglecting to use correct amounts of salts. A single example

FIGURE 8. — The upper tracing shows the effect of placing a strip of heart-muscle in cane sugar solution for two hours and 36 minutes, and then transferring it, at the point indicated by the arrow, to a solution of sugar + CaCl₂ and KCl in proportions most favorable to the development of beats. The lower control was put into cane sugar solution for two hours and 36 minutes, and then, at the point indicated by the arrow, into 89 c.c. cane sugar solution + 11 c.c. NaCl solution, in which it beat without calcium or potassium salts being present.

will illustrate the method. Heart-strips were put into cane sugar and from this transferred to a solution consisting of 89 c.c. cane sugar + 10 c.c. of a 0.26 per cent calcium chloride solution + 1 c.c. of a 3 per cent potassium chloride solution.

This solution fulfilled the physical requirements of Howell's theory, and had the proportions of calcium and potassium which were most favorable according to Greene; yet the strip would not beat, though permitted to stand twenty-four hours, at the end of which time it was dead. A control strip in a solution of 89 c.c. cane

[1] GREENE: *loc. cit.*, p. 125.

sugar + 11 c.c. of sodium chloride beat rhythmically for four hours. The rhythm was rapid and a little irregular. (See Fig. 8.)

I found that the most favorable proportions of the salts mixed with crystalline dextrose or with glycerine were also unable to start a rhythm. But the control strips treated with sodium chloride always contracted rhythmically. In short, calcium and potassium alone or in combination cannot start rhythmic beats, while sodium chloride always succeeds.

VIII. Conclusions.

1. The results of Loeb's experiments on rhythmic contractions in striped muscle and Gonionemus tissue apply very closely to the ventricular tissue of the turtle.

2. Sodium and not calcium is the stimulus for rhythmic contractions in the heart.

3. A pure sodium chloride solution has an injurious effect on heart-tissue.

4. Calcium and possibly potassium salts improve the rhythm by neutralizing this injurious action.

5. Heart-strips will not beat rhythmically in solutions of non-conductors.

THE RELATION OF THE DEPRESSOR NERVE TO THE VASOMOTOR CENTRE.[1]

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CONTENTS.

INTRODUCTION.

IT is known that the nerve cells in the bulbar vasomotor centre send their axis-cylinder processes to many subsidiary cells, through which the bulbar discharges may reach the most distant peripheral structures. It is known, too, that all parts of the body are connected with the vasomotor centre by afferent nerves. Into this centre flow countless impulses, a never-interrupted stream. Yet the problem of the relation of the afferent fibres to the individual cells of the centre has not been formulated with much precision, nor have satisfactory quantitative methods been employed for inquiring into this relation.

The older conception of the nerve centre as a narrowly circumscribed compact group of nerve cells tended to sink the individuality of the cell. The more modern conception of a number of cells associated in function rather than grouped closely in space leads directly to the consideration of the cells as individuals. This individual conception suggests the thought that the same principle of division of labor which set aside the bulbar vasomotor cells for the control of the diameter of all the blood-vessels in the body may further have given the control of particular regions into the special keeping of certain of these cells. It is true that the space occupied by the bulbar vasomotor centre is not very great, when measured macroscopically, but relatively it is great enough to harbor an im-

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[1] A summary of these experiments was printed in the Proceedings of the American Physiological Society. This journal, 1900, iii, p. xxiv.

mense number of nerve cells without a degree of crowding that would
throw doubt on the mechanical possibility of groups functionally
separate.

The importance of this problem will be obvious when the reader
reflects that if the afferent vasomotor fibres influence all the bulbar
cells alike, the bulbar centre can have no part in the distribution of
the blood to the several regions and organs of the body, but can act
merely to lower or raise the general blood pressure. The local dis-
tribution of the blood would then be the function of the spinal or
sympathetic vasomotor neurons, or perhaps to some extent of the
blood-vessels themselves.

The most conspicuous afferent path to the vasomotor centre is
furnished by the depressor nerve, and the central relations of this
nerve are naturally to be investigated first. An additional reason
for choosing the depressor nerve is found in certain experiments
made by Cyon and Ludwig[1] which bear upon the problem, though
they do not solve it; nor do they indicate, indeed, that the problem
stated here was clearly recognized.

The experiments of Cyon and Ludwig are described in that beau-
tiful example of the genius of investigation in which the discovery of
the depressor nerve is recorded. It will be remembered that Cyon
and Ludwig, after demonstrating that the excitation of the central
end of the depressor nerve caused a great fall in the general blood-
pressure, excluded a change in the heart beat as the cause of the fall
in pressure and thus reached the conclusion that the cause lay in a
reduction of the peripheral resistance. Knowing the large part which
the abdominal vessels play in the peripheral resistance, they sus-
pected that the depressor produced its effect especially by their dila-
tation. This explanation they put to experimental proof in two
ways. First, the depressor nerves were stimulated after the section
of both splanchnic nerves; the blood-pressure was observed to fall
only 2.5 mm. of mercury (from 31.5 to 29 mm.) Secondly, the
splanchnic area was excluded by the compression of the aorta just
below the diaphragm, and the depressor then stimulated. In one of
the experiments of this latter sort, the blood-pressure, which had
risen on compression of the aorta from 47 to 105 mm. of mercury,

[1] Cyon, E., and Ludwig. C.: Arbeiten aus der physiologischen Anstalt zu
Leipzig vom Jahre 1866, pp. 128–149. Also: Berichte der mathematisch-physikal-
ischen Classe der königlichen Sächsischen Gesellschaft zu Leipzig, 1866, xviii. pp.
307–328.

was not lowered by depressor stimulation; in the other, it rose on compression from 42 to 143 mm., and, on excitation of the depressor, fell to 134 mm. Both these procedures seemed to Cyon and Ludwig to demonstrate that the depressors act almost entirely through the splanchnic nerves upon the abdominal vessels. With this statement they leave the question.

The methods here employed by these distinguished investigators are not beyond criticism. In the first experiment the simple stimulation of the depressor before and after the section of both splanchnic nerves is used to measure quantitatively the difference between the effect of the depressor on the abdominal vessels and its effect on all the other blood-vessels. But the two measurements compared are not made from the same level. The section of the splanchnic nerves reduced the blood-pressure usually from 30 to 50 per cent. The blood-vessels, except in the abdomen, were thus comparatively empty when the depressor was stimulated. Consequently their dilatation, on stimulation of the depressor, could not produce so great an effect as if they had been normally full. The second experiment is still less satisfactory. The compression of the aorta immediately beneath the diaphragm excludes from depressor action not only the abdominal vessels but those of the lower part of the trunk and the hind limbs — more than half the body. The regions still accessible to the depressor are the head, neck, thorax, and fore-limbs. But the cranial and thoracic viscera are so poorly supplied with vasomotor nerves that they are frequently declared to have none, and it is known that the dilatation of the vessels of the head and neck does not very materially affect the general blood-pressure. Evidently the experiment cannot properly be used to compare the action of the depressor on the splanchnic area with its action on all the remaining vascular areas. In short, Cyon and Ludwig do not prove that the fall in blood-pressure on depressor stimulation is due chiefly to the dilatation of the abdominal vessels. They prove merely that by section of the splanchnic nerves the blood-pressure is lowered almost as much as by stimulation of the depressor.

Bayliss[1] secured plethysmographic tracings showing vascular dilatation in the limbs and in the tongue and ear. He does not attempt to estimate the relative dilatation of the abdominal and extra-abdominal vessels.

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[1] BAYLISS, W. M.: Journal of physiology, 1893, xiv. pp. 303-325.

The Method.

The method devised for the present investigation consists of (1) the determination of the fall in general blood-pressure produced by the stimulation of the depressor nerves in animals in which the splanchnic nerves are prepared for experimentation but are still connected with the vasomotor centre; (2) the removal of the splanchnic area from the control of the vasomotor centre by the section of the splanchnic nerves; (3) the restoration of the general blood-pressure to the normal level, after the fall which the section of the splanchnics causes, by the stimulation of the peripheral ends of the splanchnic nerves or by the injection of normal salt solution into the jugular vein; (4) the stimulation of the depressors while the blood-pressure is maintained near the normal height and the splanchnic area is excluded by the previous section of its nerves.   The comparison of the fall obtained by the second stimulation (the blood-pressure being normal and the splanchnic area excluded), with that obtained while the splanchnic nerves were still unsevered, will determine the relative parts taken by the splanchnic area and the remaining vascular areas in the depressor effect.   If it be found that depressor stimulation lowers the blood-pressure as much after splanchnic section as before it, the depressor certainly does not produce its effect by means of special connections with the splanchnic area through the bulbar cells; and if a special connection does not exist in the case of the splanchnic area, it is probable that there is no such special connection with any vascular area, or, in other words, that the depressor nerves act on all the bulbar vasomotor cells alike.

The animals used were rabbits.   They were anæsthetized with a mixture of three parts ether and one part 96 per cent alcohol.   Two series of experiments were performed: in the first, the blood-pressure was restored to normal after the section of the splanchnics by the stimulation of the peripheral ends of these nerves; in the second, the blood-pressure was restored to normal after the section of the splanchnics by the injection of normal salt solution into the jugular vein.

In the first series of experiments, the anæsthetized rabbits were tracheotomized, and both depressor nerves were isolated, ligated with silk, and severed on the cardiac side of the ligature.   A cannula was tied in the left carotid artery.   The abdomen was opened in the

linea alba from the ensiform cartilage nearly two-thirds of the distance to the symphysis. Two hooks on each side were passed through the edges of the wound and drawn up by threads passed over bars placed alongside the animal and ten to fifteen centimetres above it. The abdominal walls formed thus a deep oblong cavity, at the bottom of which lay the intestines. The rabbit board was inclined to the right, in order that the left splanchnic nerve might be more easily reached near the suprarenal body. The nerve was grasped with very small "bull-dog" forceps and shielded electrodes passed around it distal to the forceps. The nerve was then severed on the proximal side of the forceps, which prevented the stump from slipping through the hard rubber shield of the electrodes. As soon as the splanchnic nerve was severed, the foot of the rabbit board was raised about five centimetres higher than the head, to prevent anæmia of the brain from the filling of the relaxed abdominal vessels. The rabbit board was now tilted towards the left, artificial respiration was begun, and the central tendon of the diaphragm was incised so that the right splanchnic nerve could be reached. This was secured and severed in the same manner as the left nerve. During these several procedures the intestines were kept covered with pads of absorbent cotton wet with warm 0.8 per cent sodium chloride solution; they were never touched by the fingers directly, but were pressed gently out of the way with the cotton pads wet in normal salt solution. After the preparation of the splanchnic nerves, the wound was closed with a few stitches and the abdomen covered with a thick layer of dry cotton.

The carotid cannula was connected with a Hürthle membrane manometer and the rabbit board placed on a box high enough to bring the artery level with the chamber of the manometer. The membrane manometer was graduated by means of a mercury column in the usual way; the graduation scale is reproduced in Fig. 4, page 295; the writing point returned from 100 mm. mercury pressure accurately to the zero abscissa when the chamber of the manometer was placed in communication with the atmospheric air. A separate Du Bois-Reymond inductorium was used to stimulate each of the splanchnic and each of the depressor nerves. The inductoriums were arranged in two pairs. In each pair the primary coils were connected through a double key (Pohl's commutator, without cross-wires), with two Daniell cells in such a way that on turning the cradle the current from one cell passed through one inductorium

and the current from the other cell passed simultaneously through the other inductorium. The secondary coils were connected with the splanchnic nerves by means of the shielded electrodes and with the depressor nerves by ordinary electrodes. In the primary circuit of one of the depressor coils, an electromagnetic signal was introduced to mark the duration of depressor stimulation. The beginning and the end of splanchnic stimulation were marked on the drum by hand. Three persons took part in each experiment; one managed the stimulating currents and the graphic record, while each of the others held an electrode against the central segment of a depressor nerve raised in the air by means of a silk thread. When all was ready, the kymograph drum was allowed to revolve and after a few moments during which the low blood-pressure following splanchnic section was recorded the peripheral ends of the divided splanchnic nerves were stimulated continuously. The blood-pressure was raised by this splanchnic excitation to almost the normal level, and during its maintenance at this level the central ends of both depressor nerves were stimulated for a period sufficient to bring out the full depressor effect. Then the stimulation of the depressors was stopped while the splanchnic stimulation went on. The complete division of the splanchnic nerves was verified in each case by post-mortem examination.

The manipulation of the intestine necessary in preparing both splanchnic nerves for stimulation is much greater and more prolonged than where the nerves are simply severed. The shock of the former operation and the inevitable loss of tone in the vasomotor system make it difficult to secure maximum vasomotor effects. A second series of experiments was accordingly made. In these, the splanchnic nerves were severed with the least possible disturbance of the viscera and the blood-pressure was restored to normal by the injection of warm normal salt solution into the jugular vein. The depressor nerves were then stimulated as before.

THE EFFECT OF DEPRESSOR STIMULATION IN ANIMALS IN WHICH THE SPLANCHNIC NERVES ARE PREPARED BUT ARE NOT SEVERED.

In considering the results gained with this method it is obvious that the effect on blood-pressure of depressor stimulation with un-severed splanchnic nerves must first be examined, for the present

inquiry consists essentially in comparing the depressor action before and after the separation of the splanchnic area from the vasomotor centres. Obviously, too, the fall in blood-pressure obtained with animals uninjured except by the relatively slight operation of section of the depressors and the placing of a cannula in an artery will not serve for this comparison. Allowance must be made for the disturbance of the vasomotor system consequent upon the manipulation of the intestine in preparing the splanchnic nerves for experimentation. It is necessary to determine the effect of depressor stimulation in animals in which the splanchnic nerves have been prepared but have not been severed. The following extract from the protocol of October 3, 1899, is evidence of the serious impairment of vasomotor function which may follow the manipulation of the intestine.

Experiment Oct. 3, 1899. — The depressor nerves were prepared in a rabbit anæsthetized with ether and alcohol. The carotid blood-pressure was recorded by means of a mercury manometer. The central ends of both depressor nerves were stimulated simultaneously. The blood-pressure fell from 106 to 76 mm. Hg (28 per cent). On repeating the stimulation, the pressure fell from 112 to 76 mm. (32 per cent). Threads were now placed about the splanchnic nerves, but the nerves were not severed. This manipulation caused the blood-pressure to fall to 64 mm. On stimulation of both depressors, there was a further fall to 50 mm. (22 per cent).

Observations accordingly were made to determine the degree to which the depressor nerves can lower blood-pressure in animals in which the abdominal viscera have been disturbed by the preparation of the splanchnic nerves. It should be stated that these experiments were done after long practice in the preparation of the splanchnic nerves, and that every precaution against rough handling was taken. The results are presented in Table I (p. 297).

In the experiment of October 14, which is one of those included in Table I, the blood-pressure, which had fallen to about 60 mm. in consequence of the preparation of the splanchnic nerves, rose suddenly without any apparent cause to about 120 mm. The depressors were at once stimulated and the pressure fell to about 55 mm. (Fig. 1). On repeating the stimulation the blood-pressure fell from 120 mm. to 60 mm. (50 per cent), but after the stimulation the pressure returned only to 105 mm., and soon after fell rapidly in such a way as to make a continuation of the experiment imprudent. It seems best

to mention this experiment particularly, although the exceptional character of the observation unfits it for use as a basis of comparison. So large a fall in blood-pressure, after the preparation of the splanch-nic nerves, was not observed again, either before or after their separa-tion from the vasomotor centre.
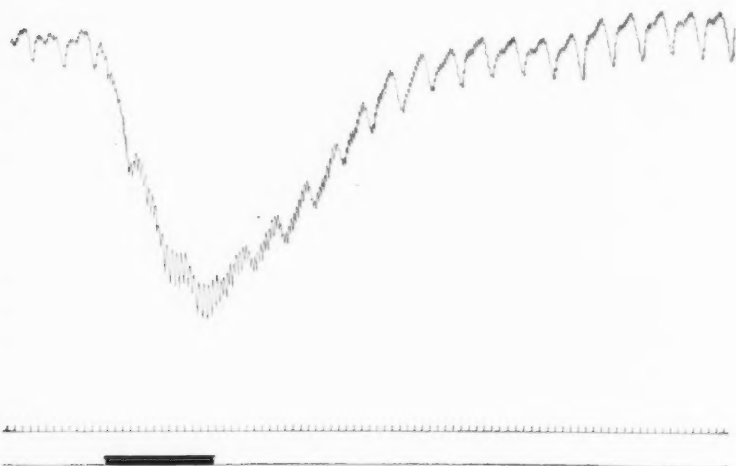
FIGURE 1. — October 14, 1899.   A curve of blood-pressure in the carotid artery recorded by a mercury manometer.   The middle line marks the time in seconds. The lowest line marks the atmospheric pressure; it was drawn by the writing point of an electro-magnetic signal in the primary circuit of one of the stimulating induct-oriums.   The broad black band records the simultaneous stimulation of both depressor nerves.   The blood-pressure fell from about 120 mm. to about 55 mm. (54 per cent).

It appears from Table I that in animals in which a thread has been placed around both splanchnic nerves the stimulation of the depressor nerves causes a fall in blood-pressure usually of from 35 to 40 per cent.   With this must be compared the effect of depressor stimulation when the splanchnic nerves are separated from the vaso-motor centre and the blood-pressure maintained near the normal level.

THE EFFECT OF DEPRESSOR STIMULATION AFTER THE SEPARATION OF THE SPLANCH-NIC AREA FROM THE VASOMOTOR CENTRE.

It has already been stated that the blood-pressure was raised after section of the splanchnic nerves by the stimulation of their peripheral ends, or by the injection of normal salt solution into the jugular vein. The experiments in which the pressure was raised by stimulation will be presented first. These experiments are illustrated by Fig. 2, from an experiment performed April 7, 1899.

In this figure arrows mark respectively the beginning and the end of stimulation of both splanchnic nerves. The upper curve records the blood-pressure in the carotid artery, registered by a Hürthle membrane manometer. The lower curve was drawn by an electro-magnetic signal, the writing point of which lay in the line of atmospheric pressure; the heavy black line marks the vibration of the signal throughout the simultaneous stimulation of both depressor nerves. On stimulation of the splanchnic nerves the blood-pressure rose from 45 to 58 mm. of mercury; by depressor stimulation it was lowered to 35 mm.; a few moments after depressor stimulation it rose to 54 mm.; on ceasing the stimulation of the splanchnics the blood-pressure sank gradually to its former level of 45 mm. Thus depressor stimulation, after the splanchnic nerves were separated from the vasomotor centres, caused a fall of forty per cent in the general blood-pressure.

The results of this series of experiments are shown in Table II (p. 298).

The table shows that the stimulation of the depressor nerves after the separation of the
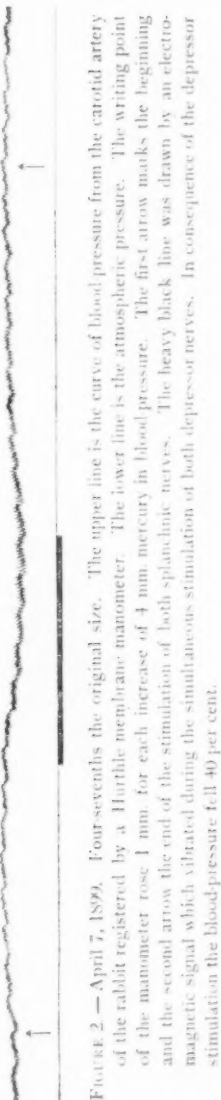
FIGURE 2.—April 7, 1899. Four-sevenths the original size. The upper line is the curve of blood-pressure from the carotid artery of the rabbit registered by a Hürthle membrane manometer. The lower line is the atmospheric pressure. The writing point of the manometer rose 1 mm. for each increase of 4 mm. mercury in blood pressure. The first arrow marks the beginning and the second arrow the end of the stimulation of both splanchnic nerves. The heavy black line was drawn by an electro-magnetic signal which vibrated during the simultaneous stimulation of both depressor nerves. In consequence of the depressor stimulation the blood-pressure fell 40 per cent.

splanchnic area from the vasomotor centre lowers the blood-pressure from 30 to 40 per cent.

The placing of shielded electrodes about the splanchnic nerves is a much more difficult operation than the simple section of the nerves, and the disturbance of the vasomotor function is correspondingly great. It was decided, therefore, to make a second series of experiments, in which the pressure should be raised by injecting into the jugular vein warm 0.8 per cent sodium chloride solution. The results are shown in Table III (p. 299). These measurements entirely confirm those presented in Table II.

The protocol and one curve from the experiments of October 21 and October 26 will sufficiently illustrate the work summarized in the tables.

Experiment Oct. 21, 1899. — In a rabbit anæsthetized with a mixture of ether and alcohol both depressor and both splanchnic nerves were prepared in the manner already described (page 287). A thread was passed around each splanchnic nerve. Great pains were taken not to injure the intestines. The blood-pressure was recorded by a Hürthle membrane manometer connected with a cannula in the carotid artery. A cannula was also placed in the right jugular vein. The central ends of both depressors, raised in the air on silk threads, were stimulated simultaneously. The blood-pressure fell from 80 to 60 mm. mercury (25 per cent). On repeating the stimulation (with somewhat too brief an interval) the pressure fell from 75 to 60 mm. (20 per cent). The splanchnic nerves were now torn through by means of the threads which had been passed around them. The blood-pressure thereupon fell to 60 mm. Through the cannula in the right jugular vein 0.8 per cent sodium chloride solution at 38° C. temperature was injected until the blood-pressure rose to 85 mm. The depressors were then stimulated again, with the following result : —

Blood-pressure before stimulation of depressor nerves.	Lowered by stimulation to	Fall per cent.
85 mm.	65 mm.	24
85	58	32
88	60	32
85	58	32

The artificial respiration, which had been begun during the preparation of the right splanchnic nerve, was now discontinued during the brief period required for renewed stimulation. The rabbit did not become dyspnœic. The oscillations of the blood-pressure of course disappeared, thus making the

reading of the blood-pressure easier. The stimulation of both depressors now gave : —

Blood-pressure before depressor stimulation.	Lowered by stimulation to	Fall per cent.
86 mm.	60 mm.	30
85	60	29
90	60	33
88	55	37
90	57	37

(The last of these measurements is shown in Fig. 3.)

Fearing that the fall in blood-pressure in the last two stimulation periods might be thought to be due in part to the escape of the stimulating current to the vagus, both vagi were severed between the chest and the point at which the depressors were stimulated. As in all experiments, the central ends of the severed nerves were held on a black silk thread well into the air. On renewing the stimulation the blood-pressure fell from 90 to 60 mm. (33 per cent). The post mortem examination showed that the splanchnic nerves had both been severed.

One of the records made in this experiment is reproduced in Fig. 3.
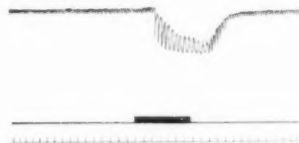
FIGURE 3. — October 21, 1899. Original size. The uppermost line is the curve of blood-pressure in the carotid artery registered by a Hurthle membrane manometer. The middle line is atmospheric pressure; this line was drawn by the writing point of an electro-magnetic signal, which recorded with a broad black line the duration of depressor stimulation. The lowest line marks the time in seconds. Both splanchnic nerves were severed. The blood-pressure was raised after this section by the injection of 0.8 per cent NaCl solution into the jugular vein. On simultaneous stimulation of both depressor nerves the blood-pressure fell from 90 mm. to 57 mm. (37 per cent).

In this figure, the uppermost line was drawn by a Hurthle membrane manometer connected with the carotid artery. The middle line records the atmospheric pressure. It was drawn by the writing point of an electro-magnetic signal placed in the primary circuit of one of the inductoriums used to stimulate the depressor nerves. The heavy black band upon this line marks, therefore, the simultaneous excitation of both depressor nerves. The lowest line marks

the time in seconds. Both splanchnic nerves had been severed. The blood-pressure had been raised after splanchnic section to 90 mm., by the injection of warm 0.8 per cent sodium chloride solution into the right jugular vein. On stimulating both depressor nerves, the blood-pressure fell to 57 mm. (37 per cent).

The experiment of October 26 is equally instructive.

Experiment Oct. 26, 1899. — In a rabbit prepared as in the experiment of October 21 the depressor nerves were stimulated while the splanchnic nerves were still uncut.

Blood-pressure before stimulation of depressor nerves.	Lowered by stimu- lation to	Fall per cent.	Remarks.
57 mm.	35 mm.	39	The artificial circulation was
68	48	29	interrupted, and the stimu-
75	38	49	lation repeated.
57	35	39	Artificial respiration was
68	48	29	stopped during stimulation.
75	38	49	This record is reproduced in Fig. 4.

Both splanchnic nerves were now torn through by means of the threads which had been placed around them. The blood-pressure thereupon fell to 52 mm. By the injection of warm 0.8 per cent sodium chloride solution into the right jugular vein the blood-pressure was raised again. The stimulation of the depressor was repeated, the artificial respiration being stopped during each stimulation period.

Blood-pressure before stimulation of depressor nerves.	Blood- pressure lowered to	Fall per cent.	Remarks.
80 mm.	50 mm.	38	
65	40	38	
87	55	37	More salt solution injected.
70	50	29	With artificial respiration.
70	50	29	Without artificial respiration. (Note that suspending the artificial respiration during the period of observation does not impair the method.)
76	50	34	
80	48	40	This record is reproduced in Fig. 5.
84	53	37	

Figure 4 is a photographic reproduction of the alteration in the blood-pressure curve produced by the simultaneous stimulation of both depressor nerves while the splanchnic nerves were still connected with the vasomotor centre.

In this figure the uppermost line is the curve of blood-pressure in the carotid artery. It was drawn by a membrane manometer the
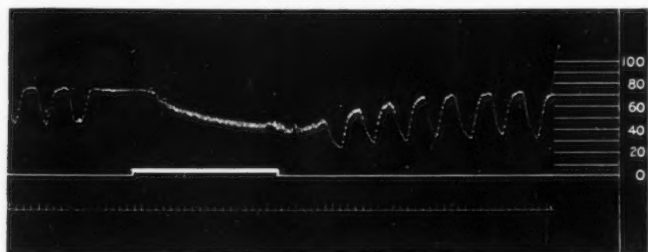
FIGURE 4.— October 26, 1899. Original size. The uppermost line was drawn by a membrane manometer connected with the carotid artery. The graduation scale of this manometer is reproduced on the right. The middle line marks atmospheric pressure; it was drawn by the writing point of an electro-magnetic signal which also recorded, by a white band, the simultaneous stimulation of both depressor nerves. The lowest line marks the time in seconds. The artificial respiration was suspended during the observation. The splanchnic nerves had been prepared for experimentation, but were not yet separated from the vasomotor centre. On stimulating the depressors the blood-pressure fell from 75 mm. to 38 mm. (49 per cent).

graduation scale of which appears on the right of the figure. The middle line marks the atmospheric pressure; the broad white line records the simultaneous stimulation of both depressor nerves. The lowest line gives the time in seconds. The artificial respiration was suspended for a brief period during the observation. Comparative observations, one of which is recorded in the protocol of October 26, show that this procedure does not impair the usefulness of the method. On stimulating the depressor nerves the blood-pressure fell from 75 mm. to 38 mm. (49 per cent). This result is to be compared with that shown by Fig. 5.

In this figure, as in Fig. 4, the uppermost line is the curve of blood-pressure in the carotid artery, the middle line records the atmospheric pressure and the stimulation of the depressor nerves, and the lowest line records the time in seconds. Both splanchnic nerves had been severed. The blood-pressure was raised after the section of the splanchnic nerves by the injection of 0.8 per cent

sodium chloride solution into the jugular vein. On stimulating the depressor nerves the blood-pressure fell from 80 mm. to 48 mm. (40 per cent). Thus the fall secured after the separation of the splanchnic nerves from the vasomotor centre was almost as great as that obtained before these nerves were severed.

It appears, therefore, from these numerous experiments that the stimulation of the depressor nerve can lower the blood-pressure as much, or exceptionally almost as much, when the abdominal vessels are separated from the vasomotor centre by the section of the splanchnic nerves as when this connection is intact, provided only

FIGURE 5. — October 26, 1899. Original size. As in Fig. 4, the uppermost line records the carotid blood-pressure, the middle line the atmospheric pressure and the stimulation of the depressor nerves, the lowest line the time in seconds. Both splanchnic nerves had been severed. The blood-pressure was raised by the injection of warm normal salt solution into the jugular vein. On stimulating the depressor nerves, the blood-pressure fell from 80 mm. to 48 mm. (40 per cent).

that the general blood-pressure after section of the splanchnic nerves be raised high enough to enable the depressor nerves to act with power. The few exceptional cases (for example, Fig. 5), in which the depressor stimulation failed to lower the blood-pressure to quite the same degree after the section of the splanchnic nerves as before section, may reasonably be explained by the unavoidable shock of the operation, and the relatively crude method of restoring the level of the blood-pressure.

CONCLUSION.

There is no sufficient evidence that the depressor nerve forms a special connection with the cells which control the vasomotor fibres of the splanchnic nerves. It is probable that the depressor nerves

connect in the same way with all the cells in the bulbar vasomotor
centre, and there is no reason to suppose that other afferent vaso-
motor nerves differ in this respect from the depressor nerve. Afferent
vasomotor fibres would thus influence all the bulbar vasomotor cells
alike, and the bulbar centre would have no part in the distribution
of the blood to the several organs and regions of the body. The
bulbar centre would act merely to raise or lower the general blood-
pressure.

TABLE I.

Showing the fall in blood-pressure produced by the simultaneous stimulation of both
depressor nerves in rabbits in which the splanchnic nerves were prepared but were not
severed.

BLOOD-PRESSURE IN MM. HG.

Date (1899).	Before stimulation of both depressors	Lowered by stimulation to	Fall (per cent).	Remarks.
Oct. 3	64 mm.	50 mm.	23	Thread around each splanchnic ; mercury manometer.
" 4	60	40	33	Thread around left splanchnic ; right nerve not
	70	47	33	prepared ; mercury manometer.
	54	38	30	
" 6	110	64	42	Thread around each splanchnic ; artificial respiration ;
	107	67	37	mercury manometer.
	110	70	36	
" 14	60	41	32	Thread around each splanchnic ; artificial respiration ;
	66	41	38	mercury manometer.
	120	55	54	
" 21	80	60	25	Thread around both splanchnics ; Hürthle manometer.
	75	60	20	
" 26	57	35	39	Thread around both splanchnics ; artificial respiration.
	68	48	29	
	75	38	49	The rise to 75 mm. was due to stopping the artificial respiration.

TABLE II.

The effect on blood-pressure of depressor stimulation before and after the separation of the splanchnic area from the vasomotor centre. The pressure after section of the splanchnic nerves was raised by the stimulation of their peripheral ends.

BLOOD-PRESSURE IN MM. OF MERCURY.

Date (1899).	After section of both splanchnic nerves.	On stimulation of the peripheral segments of the splanchnic nerves.	On depressor stimulation.	Fall per cent on depressor stimulation.
March 20	55 mm.	85 mm.	60 mm.	30
	60	105	80	24
" 22	70	78	55	30
" 24	25	35	25	30
April 1	65	100	75	25
" 6	50	70	40	43
	45	70	48	31
	48	65	38	41
" 7	45	58	35	40
	45	52	33	37
" 8	38	60	48	20
	44	65	52	20
	52	70	50	29
	42	58	44	24

TABLE III.

The effect on blood-pressure of depressor stimulation before and after the separation of the splanchnic area from the vasomotor centre. The pressure after section of the splanchnic nerves was raised by the injection of warm 0.8 per cent sodium chloride solution into the jugular vein.

BLOOD-PRESSURE IN MM. OF MERCURY.

Date (1899).	After section of both splanchnic nerves.	On stimulation of the peripheral segments of the splanchnic nerves.	On depressor stimulation.	Fall per cent on depressor stimulation.
Oct. 7	50 mm.	90 mm.	53 mm.	41
" 17	50	110	75	32
		125	85	32
" 21	60	85	65	24
		85	58	32
		88	60	32
		85	58	32
		86	60	30
		85	60	29
		90	60	33
		88	55	37
		90	57	37
		90	60	33
" 23	52	60	45	25
" 26	52	80	50	38
		65	40	38
		87	55	37
		70	50	29
		70	50	29
		76	50	34
		80	48	40
		84	53	37

THE INFLUENCE OF CHANGES IN TEMPERATURE UPON NERVOUS CONDUCTIVITY AS STUDIED BY THE GALVANOMETRIC METHOD.

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CONTENTS.

I. INTRODUCTION.

IN general each tissue has its upper and its lower lethal temperature, and its thermal optimum. Nerve tissue is sensitive to changes in temperature, as we may observe in studying the terminal organ of a nerve or the galvanometric response.

By means of a certain degree of heating or cooling we are enabled to block the nerve impulse, without necessarily killing the nerve.

The velocity with which the nerve impulse is propagated depends upon the temperature of the nerve, being greater at higher temperatures.

The power to respond to external stimuli is also influenced by the temperature; a warmed nerve is more capable of replying to a short stimulation than a cooled nerve.

Conductivity of a nerve, or the power to respond to its own internal stimulus, is affected by the temperature; for, if the nerve impulse passes through a heated area, it is increased; if through a cooled area, it is diminished.

That the nerve impulse is increased in passing through a heated area, has been observed, so far as I know, only in the case in which

the end effects of the stimulus in the organ supplied by the nerve were observed. As is well known, we have two methods of studying nervous activity: — we can take the organ supplied by the nerve, most conveniently the muscle, and observe its response; or we can employ the galvanometer or electrometer, which will indicate the differences of electric potential set up between different points of the nerve, when a nerve impulse passes. In the latter method the negative variation or action current becomes the object of observation. It has been generally accepted that this current of action is a true measure of the nerve impulse which causes it. It has been the main object of this investigation to determine whether or not the action current is increased when the nerve impulse passes through a heated area.

II. STATEMENT OF THE PROBLEM AND HISTORICAL REVIEW.

The problem may be stated schematically as follows: — If *a b c*
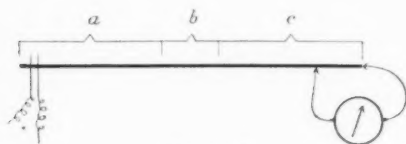
FIGURE 1.

be the nerve (Fig. 1), and the temperature of *a* and *c* be maintained constant while the temperature of *b* is raised, will the negative variation observed in the galvanometer be increased? Under similar conditions it has been shown that the muscle response following indirect stimulation is increased. If the galvanometer takes the place of the muscle, will its deflections increase, as the muscle contractions increase?

Bernstein[1] observed that when he raised the temperature of the nerve between the stimulating electrodes and the muscle, the muscular response increased. He called attention to the fact that raising the temperature of the nerve at the point stimulated would increase the electrical conductivity, an observation which evidently escaped Gotch and Macdonald.[2]

Howell[3] noted an increased vaso-constriction upon allowing the nerve impulse to pass through an area heated to 42°–47° C. Howell's, as well as Bernstein's, observations were made on mammalian tissue.

[1] BERNSTEIN: Archiv für die gesammte Physiologie, 1877, xv. p. 310.
[2] GOTCH and MACDONALD: Journal of physiology, 1896, xx, p. 247.
[3] HOWELL, BUDGETT, and LEONARD: Journal of physiology, 1894, xvi, p. 298.

Hirshberg[1] studied the problem on frog's nerve. He found that upon warming a nerve locally from 15°–30° C. and stimulating in the warmed region, the secondary coil had to be shoved farther out from the primary for a minimal contraction, i. e. the irritability was increased. But if he then stimulated above the warmed area, thus permitting the impulse to pass through a stretch of nerve of higher temperature, the coil had to take its original position for minimal contractions, i. e. there was no change so far as the conductivity of the nerve was concerned. This negative result might easily be explained by the comparatively low temperature (30° C.) selected.

Verweg,[2] in his experiments upon the effect of thermal changes on the duration of the monophasic action current, discovered that on passing the nerve impulse through a cooled area or stimulating in a cooled area, there was a decrease in the size of the action current, although there was no change in its duration. In these experiments he also warmed the nerve between the point stimulated and the portion led off to the galvanometer, but he does not compare the action current so obtained with that observed without warming, nor does he give the exact temperatures employed.

Boruttau[3] studied the effect of passing the nerve impulse through a cold area, and found that the negative variation persisted after the muscular response had disappeared. He also verified and extended Verweg's work, finding that the duration of the diphasic, as well as that of the monophasic action current depends only upon the temperature of the stretch of nerve included in the galvanometer circuit, not upon the stretch outside that circuit. He very aptly calls attention to the following : —

If in our Fig. 1, *a*, *b*, and *c* are all at the same temperature, say 20° C., then the impulse, started in *a* at the exciting electrodes and passing through *b*, will arrive after a certain time at the proximal electrode, causing a monophasic action current. If now the temperature of *a* and *c* remain unchanged, while *b* is cooled down to 5° C., we shall notice : (1) that the negative wave reaches the proximal electrode later than in the first case, due to the retardation in *b*; (2) that the action current is less intense, i. e. of less amplitude; (3) that the duration is unchanged, because in order to affect this, the temperature

[1] Hirshberg : Archiv für die gesammte Physiologie, 1886, xxxix, p. 75.
[2] Verweg : Archiv für Anatomie und Physiologie, 1893, p. 523.
[3] Boruttau : Archiv für die gesammte Physiologie, 1897, lxv, p. 7.

of the led-off stretch, *i. e.* the part between the galvanometer electrodes, must be changed.

If instead of cooling *b*, as Boruttau did, we warm it, we should expect: (1) the arrival of the wave of negativity at the proximal electrode to occur after a shorter interval than when the nerve was throughout at the temperature of 20° C.; (2) a greater intensity of action current; (3) no change in the duration of the monophasic action current.

Waller,[1] in studying the effect of variations of temperature upon the electrotonic currents A and K, observed that the negative variation is temporarily abolished at about 40° C.; a positive takes the place of a negative variation in consequence of a temperature raised to 40° C.

III. Division of Experiments.

The experiments composing this investigation may be best divided into two series.

The experiments of the first series were carried out in the spring of 1899, and may be grouped under two heads: — those relating to conductivity, and those relating to irritability. In these experiments no increase in the action current was observed, whether the stretch *b* or the stimulated stretch was heated. In the conductivity experiments, however, because of the small action currents observed and the nearly maximal stimulus employed, the results were not satisfactory. In addition to this a new form of non-polarizable electrode had been used, which had not proved of equal rank with the usual form. I wished to check my results, and the conductivity experiments — as these were of first importance — were therefore continued.

These latter experiments accordingly form the second series, and were performed during the past scholastic year. They may be classed under three groups: — (1) stimulation by means of induced currents; (2) condenser discharge used as stimulus; (3) the negative variation as obtained by reflex stimulation.

As a check upon the galvanometer work, I performed a number of experiments using the muscle as my indicator, but these experiments are not reported separately; if reported at all, they are incorporated with those in which the galvanometric response was observed.

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[1] Waller: Proceedings of the Royal Society, 1896, lx. p. 383.

IV. Experiments of the First Series.

Apparatus and Method. — The first method tried was that used by Gotch and Macdonald,[1] viz., allowing the nerve to lie across a thin-walled glass tube through which water of various temperatures could be passed. As it was found, however, that the nerve under these conditions would readily dry, this method was given up and another procedure adopted. The nerve at one place was allowed to dip into a small bath of normal salt solution; the bath was supplied by Mariotte's bottles so that the level of the bath remained constant. A number of experiments were obtained by this method, but it proved awkward and hard to control; moreover normal salt solution of 35°–40° C. cannot be regarded as an indifferent liquid.

The method finally adopted as most satisfactory was the threading of the nerve through a tunnel about which water circulated. The tunnel, about 10 mm. long, consisted of a small German silver tube let through a larger tube of the same material. The larger tube was Λ-shaped and the smaller tube pierced it at the angle. Through the larger tube water flowed from bottles placed upon an elevated stand. This form of temperature tunnel is used by Professor Howell.

The inside of the tunnel was at first coated with paraffin, then a thin-walled small glass tube was inserted which just fitted the smaller metallic tube, thus making a glass-walled tunnel. This arrangement was of advantage in that the nerve did not come into direct contact with the metal.

A small moist chamber was used consisting of the bottom of a preparation dish which was inverted and rested upon an ebonite base. Through the base projected the angle of the Λ-shaped tube containing the tunnel. A hole was drilled in the bottom of the dish, through which, when the dish was turned upside down, a thermometer was inserted to determine the temperature of the chamber.

The non-polarizable electrodes were also inserted through the base into the chamber. They deserve a word of mention, for they differed from the usual type of non-polarizable electrodes. These electrodes were patterned after those recommended by Ostwald.[2]

[1] Gotch and Macdonald: Journal of physiology, 1896, xx, p. 252.

[2] Ostwald: Hand- und Hulfsbuch, p. 258.

At Professor Howell's suggestion[1] they were used in this first series of experiments.

The substances composing the electrodes are mercury, calomel, and 0.6 per cent NaCl. The electrodes are best made by fusing a platinum wire into a glass tube of convenient size, thoroughly cleaning the tube and the piece of platinum inside by boiling in a solution of bichromate of potassium in dilute sulphuric acid, washing with tap water, then distilled water, and finally drying by alcohol and ether, when the components are put in. The tube is filled to within 3 or 4 mm. of the top with pure mercury, then a layer of calomel is added. The addition of the calomel can be most conveniently effected by having it under normal salt solution in a bottle, and taking it up along with some salt solution by a pipette (Fig. 2).

All the components of the electrode should be pure, and the tube should be kept erect, precautions being observed against shaking the mercury about.

NaCl 0.6 %
HgCl
Hg
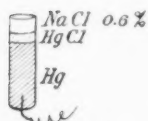
FIGURE 2.

If these electrodes are properly made they are quite isoelectric. In a few experiments made in the spring of 1899 they did not turn out to be as non-polarizable as the amalgamated zinc-$ZnSO_4$ electrode of DuBois-Reymond. They have, however, the advantage of serving for a long time, provided evaporation is avoided, without requiring to be remade. One pair, used twenty days, remained throughout quite isoelectric; the calomel and normal salt solution were, however, renewed.

A very recent communication from Oker-Blom[2] describes another form of the same electrode. I think the form used in this laboratory is the more convenient.

As stimulating electrodes platinum points were employed. They were connected with the secondary coil of an ordinary inductorium of the horizontal type. The primary coil was fed by an Edison-Lalande cell and, to equalize the make and break shocks, the Helmholtz modification was used.

The stimulus employed in these experiments of the first series was throughout faradic, but the strength of the stimulus expressed in secondary coil distance is not given. In the conductivity experiments a slightly submaximal stimulus was obtained, and was

[1] These electrodes were described by him at the meeting of the American Physiological Society, New York, 1899.

[2] OKER-BLOM: Archiv für die gesammte Physiologie, 1900, lxxix, p 534.

not changed during the experiment. In the irritability experiments a maximal stimulus was employed.[1]

The nerve used was the frog's sciatic, either fresh or after having been kept over night in a cool place. Having threaded the nerve through the tunnel and arranged it upon the two pairs of electrodes, the stimulus was selected, and then changes in the temperature of the stretch *b* or the stimulated stretch were begun. The flow of the water through the tube surrounding the tunnel was regulated by pinch-cocks, and by mixing the cold and hot supplies in proper proportions the desired temperature could be obtained. Stimulation of the heated stretch was secured by placing in the heating tunnel two little coils of platinum wire, which served as stimulating electrodes. No precautions were taken against a rise in the electric conductivity of the stimulated stretch when it was heated, because there was no increase in the action current even with the existing conditions.

The stimulated and led-off stretches were widely separated.

In order to prevent drying of the nerve a small piece of sponge was fastened to the inside of the chamber and kept moist. This moist chamber was so small that its temperature could be kept fairly constant by placing on top of it a small crucible filled with warm water when the temperature of the water around the tunnel fell, with ice-water when the temperature rose. Thus by putting on and taking off the crucible the temperature of the chamber was kept constant. By means of vaseline around the base the chamber was rendered air-tight.

Results of Conductivity Experiments. — The results of these experiments may be briefly stated as follows : —

When the stretch *b* was warmed there was no increase in the action current up to the lethal temperature, about 47° C. On the contrary, as this temperature was approached the action current decreased, disappearing in some cases even at a temperature of 42° C.

Cooling the stretch *b* sufficiently caused a considerable decrease in the action current; a temperature of 0° C. produced a marked effect, but still in general did not block the negative variation. It was only with very low temperatures, −2° to −7° C., that the negative variation disappeared, but in these instances the stimulus was nearly maximal. Of course, at this low temperature the nerve will freeze when it is exposed too long. Such low temperatures have a

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[1] For description of galvanometer see p. 310.

deleterious effect. But if these high and low temperatures at which the negative variation disappears were not maintained too long, the action current reappeared upon removing the temperature block, in some instances in full strength.

As to the temperatures high and low, at which the action current under the conditions described and resulting from a maximal stimulus, begins to decrease, it is hard to make any definite statement, for the duration of the exposure to the change in temperature is an important factor. In general the nearer the temperature lies to the lethal, the shorter has to be the exposure in order to cause a decrease in the action current, a result which, of course, would be *a priori* expected. Temperatures between 35° and 8° C. seem to be indifferent, while above 35° and below 8° there is a decrease to a complete blocking, at 47° and −7° C. respectively.

The accepted conductivity experiments number twenty-three, of which the two in Table I (pages 324, 325) may serve as examples.

Results of Irritability Experiments. — When the stimulated stretch was heated, there was no increase of the action current produced by a maximal stimulus. At 45° or a few degrees above this temperature the stimulated area lost its power to respond to the external stimulus. . Even a temperature of 29° C. seemed in one experiment to decrease the action current.

If cooling was employed the cooled stretch still remained irritable to a maximal stimulus at 0° C., though the galvanometric response was considerably lessened; at −2° to −5° C. the response was about halved. In one experiment the temperature had to be lowered to −10° C. to cut out the action current.

The temperature limits within which there was no effect we may put at 35° to 10° C.

V. Experiments of the Second Series.

The results of the experiments of the spring of 1899 were entirely negative as to any increase in the action current when the nerve impulse passes through a warmed region. But it must be remembered that in these experiments the stimulus was almost, if not quite maximal — such a stimulus being chosen in order to get a large action current.

In the experiments of the following autumn care was taken to make the stimulus certainly submaximal, and, as the experiments pro-

gressed, to make the stimulus minimal. In this series a diversity of stimuli was employed, including induced currents, condenser discharge, and reflex stimulation.

Gotch and Macdonald[1] have shown that so far as irritability is concerned, the temperature effect varies with the *kind* of stimulus employed. Warming the stimulated point of the nerve increases the muscular response; cooling decreases it, if the stimulus be the induced current. With the condenser discharge ("prolonged," Waller[2]) cooling increases, warming decreases the contraction; the same is also true for stimulation by means of galvanic and sinusoidal currents of 0.005″ or longer duration. It was therefore of interest to see whether the nerve showed any difference in its conduction of a stimulus through areas of different temperatures according as the internal stimulus was started by an induced current or a condenser discharge.

Before passing to the description of apparatus, which differed considerably from that of the first series, it may be well to call attention to a few sources of error, and point out the precautions taken.

In galvanometric experiments, in which temperature is one of the factors, care must be taken to avoid unequal heating of the electrodes which lead to the galvanometer, otherwise, as Hermann[3] pointed out, hydrothermo-electric currents will arise and be a source of error. It will be shown in what follows that this error was guarded against.

If we heat the stretch *b* (Fig. 1) we probably are justified in assuming that the change in temperature will remain well localized in *b*, owing to the poor conductivity of the nerve tissue. To be quite sure, I have in the following experiments, for the most part at least, provided against such possible conduction.

Another probable source of error is that the heating of the stretch *b* might itself act as a stimulus and produce a negative variation; this however would be a small error even if actually occurring, for as Grützner[4] has shown, the negative variation following upon stimulation by heat is excessively small.

A source of error which caused me much trouble was the drying of the nerve in the region of higher temperature. Unless the nerve

[1] GOTCH and MACDONALD: Journal of physiology, 1896, xx. p. 282.
[2] WALLER: Journal of physiology, 1899, xxiv, Proceedings of the Physiological Society, Feb. 18.
[3] HERMANN: Archiv für die gesammte Physiologie, 1877, xiv. p. 485.
[4] GRÜTZNER: Archiv für die gesammte Physiologie, 1881, xxv. p. 253.

is very large, the evaporation that goes on immediately around the stretch *b* is sufficient to dry it unless special precautions are taken such as frequent moistening, or the enclosing of the stretch in a narrow tube. In addition to the injury arising from the drying, there is also the possibility of increased irritability in the stretch *b*, since a certain degree of evaporation, as is well known, heightens the irritability.

Errors common to all galvanometric experiments are such as arise from unipolar stimulation and electrotonic currents. The first was excluded by using strengths of current incapable of producing the phenomenon. Even with one pole of the inductorium put out of circuit, I have been unable to get unipolar action with the maximum strength of current employed. Electrotonic currents were guarded against according to Bernstein's recommendation. Invariably the stimulated stretch was separated from that which was led off, almost to the entire extent allowed by the length of the nerve; the distance between stimulating electrodes was made very small, about 2 mm.; and the iron core of the primary coil was removed, Helmholtz's modification being employed at the same time to equalize the makes and breaks as much as possible.

STIMULATION BY INDUCED CURRENTS.

Apparatus. — The galvanometer employed was a high sensibility Rowland-D'Arsonval type. One volt through 940 megohms produces a deflection of one millimetre at one metre distance, *i. e.* one millimetre scale division equals about 10^{-9} ampère. The resistance of this instrument is 2786 ohms. The galvanometer is independent of the earth's field, and can consequently be placed in any desired vertical plane. It is perfectly dead beat, and is provided with an adjustable scale, on which the deflections are read with a telescope. The laboratory in which these experiments were performed is near an electric car line, but this factor was found to have no influence upon the galvanometer. I mention this, as I have read complaints from some observers who assert that their galvanometer work is very much interfered with by neighboring car lines. The D'Arsonval type of instrument has here a great advantage.

The induction coil used was the upright style made by Petzold. The secondary coil has 10,423 turns of wire. The primary coil was fed by a storage cell having a potential difference of 2 volts between

its poles. Using this cell with 3 to 5 ohms additional resistance in the circuit of the primary coil, I found that the stimulus obtained from the secondary coil first became perceptible to the tip of the tongue, when the secondary was at a distance of about 120 mm. on the graduated scale.

A special moist chamber was constructed, consisting essentially of a hollow parallelopipedon. It was closed below by a heavy ebonite base, above by a glass top. The metallic walls were double, and through the space between them water could circulate. This arrangement prevented any serious variations in the temperature of the moist chamber during the heating of the stretch *b*. The follow-ing are the inside dimen-sions of this chamber: length, 11 cm.; width, 3 cm.; height, 3 cm.

The metallic tank fitted into a correspond-ing notch around the ebonite base. The base had two slits in it which extended almost the full length of the chamber. These slits acted as guides for the stimu-lating electrodes, the tunnel-tube, and the leading-off electrodes.

------ *Tank Supply*
-- -- *Heater* "

Cold   *Hot*
FIGURE 3.

The tunnel-tube was a modified form of that used in the first series.

In order to make sure that the heating of the stretch *b* was confined to *b* two additional tunnel-tubes were used, one on each side of the heating tube. Through these water was circulated of the same temperature as that of the water supplying the tank. At first the three tubes were separate, but subsequently they were arranged together so that they formed a tunnel about 34 mm. long. Around the middle third of the tunnel passed warm water, around the two end thirds tap water (Fig. 3). In this manner the nerve lay in a chamber having a fairly constant temperature; the temperature of the stretch *b* could be varied, but this change of temperature could not be conducted along the nerve, nor could the leading-off
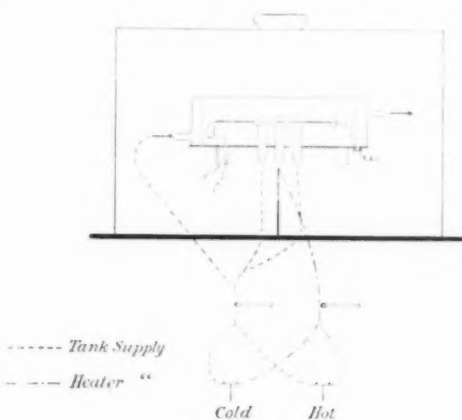
electrodes be unequally heated because of the interposed temperature tube.

The stimulating electrodes were platinum points, which had an ebonite holder fitting into the two slits of the base of the moist chamber.

In this second series, instead of the Ostwald electrodes, DuBois-Reymond's amalgamated zinc-ZnSO$_4$ electrodes with plugs of kaolin were used. Great pains were taken in their manufacture, and only those were employed which were fairly isoelectric. They were held by means of a special holder which fitted into the two slits of the base of the chamber. The two slits thus served as a track along which the stimulating electrodes, the triple tunnel-tube, and the non-polarizable electrodes could be displaced. Nine small set-screws made it possible to fix the electrodes and tubes firmly at any desired distance apart — an arrangement that proved very convenient.

The nerve chamber was supported by a rod fixed into the base of a large moist chamber. When the glass top of the latter was put in place, the small chamber occupied the centre of the space covered by the large one.

The laboratory is supplied with hot and cold water, and this supply was used instead of having bottles or tanks from which to draw the water. Small hot and cold water pipes were extended to the table standing before the galvanometer; each pipe ended in a pair of stopcocks, so that two distinct streams of water could be obtained, the temperature of each of which could be varied independently of the other. One stream supplied the metallic tank of the nerve chamber and at the same time the two end-tubes of the tunnel, while the other stream supplied the heater, *i. e.* the tube by means of which the stretch *b* was heated. The waste pipes are not indicated in the figure (Fig. 3); they led off to the sink.

By adjusting the hot and cold water stopcocks properly the temperature of either stream could be nicely regulated. Thermometers, which had been compared with a standard, were placed in the two streams and brought close together so that the readings could be easily taken. In general the temperature of the water flowing through the tank is given, as it was found that the temperature inside the chamber did not differ by more than 2 or 3 degrees from that of the water. In moderate weather tap water was used without adding any hot water. When the tap water fell below 15°, hot water was added, but in some cases cold tap water was employed; special

attention will be called to the results obtained in these cases. The temperatures were read with each reading of the action current.

In a few of the experiments a ∩-shaped glass tube was employed for raising the temperature of the stretch *b*. The limbs of the ∩ fitted into the slits in the base of the nerve chamber. Although the use of a glass tube in the first series did not seem to be successful, in the present series it was found to work very well. A wad of kaolin moistened with normal salt solution was sometimes placed about the nerve to prevent drying. In the protocols the use of the tunnel is understood unless otherwise stated.

Method and Results. — The sciatic nerve of the frog was used throughout. The frogs, which were kept in the cellar of the laboratory, were for the most part of good size. The nerves were carefully removed, a piece of the spinal column usually being left in connection with the plexus. The nerve was sectioned low down near the knee and the full length of the nerve was taken. As the action current was larger and more definite after the nerve had lain over night in a cool place upon a piece of filter paper moistened with normal salt solution, such nerves were usually selected; such a nerve is designated " kept."

Either a scissor section or a thermal section was made at the end of the nerve. The thermal section was made either with a hot glass rod or a test tube containing scalding-hot water. Since the latter method was most efficient, I shall describe it in some detail; attention to such points is very necessary in the technique of electrophysiology.

A long narrow test tube is half filled with water, and the water boiled. A portion of the end of the nerve is then brought in contact with the wall of the test tube, which is held at an angle, with the mouth away from the nerve, so that no hot water vapor will injure the rest of the nerve. A few millimetres of the end of the nerve can thus be thoroughly cooked; a scissor section is then made through the coagulated portion in case it is too long. With a millimetre or two of cooked tissue on the end of the nerve the demarcation current remains for a long time without decrease, *e. g.* in one experiment at the end of the first hour the demarcation current had fallen from 84 to 76 mm., at the end of the second hour to 59 mm. Care must of course be taken not to injure the remainder of the nerve, and also to make the thermal section clean, which is best done by holding the nerve perpendicular to the test tube.

After the non-polarizable electrodes had been prepared and put in position in the nerve chamber, a ligature of silk thread was passed around the distal end of the nerve, and tied to a thin piece of straw which served as a needle for threading the nerve through the tunnel. In order to accomplish this most easily the tank was removed from its base. The proper precautions were taken for moisture by providing a liberal supply of wet filter paper.

After the nerve was in position, the water supply was turned on and adjusted. We may call the stream flowing through the tank and the two tubes on either side of the heater the "tank supply," the current passing through the heater the "heater supply." Both streams were copious, so that the readings of the thermometers (indicated in Fig. 3) represented very closely the temperature of the water passing around the tunnel. The demarcation current was read (no compensation was used), and then the nerve was stimulated with various strengths of current. After a stimulus of a certain intensity had finally been selected, the variations in the temperature of the stretch *b* were begun. These
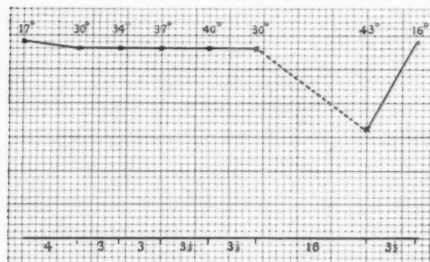
FIGURE 4. — Curve for strong stimulation. From an experiment of Nov. 17. Fresh nerve; demarcation current at beginning of experiment = 88 mm.; demarcation current at end of experiment = 66 mm. Ordinates express strength of action current; two ruled divisions are equal to 1 mm. of scale deflection; temperature of stretch *b* given above; intervals between observations, in minutes, along abscissa.

variations were for the most part sudden, *e. g.* from 20° to 35° or 40° C., because of the rapidity with which the nerve dried when exposed to high temperatures. As to the temperatures of the stretch *b* a word should be said. It will be noticed in the reports of the experiments that the temperatures producing the same effects differ somewhat. This is due to the fact that the method varied a little; for, as already mentioned, the tunnel was at first divided into three parts, which, to eliminate the danger of drying, were subsequently united. In this united condition the two cooler streams tended to counteract the warm stream, thus necessitating an elevation of the temperature of the latter.

Upon consulting the accompanying typical curve for a strong stimulus, *i. e.* a stimulus producing almost or quite a maximal galvanometric response, it will be seen that there is no increase in the action current as the temperature of the stretch *b* is raised (Fig. 4) ; no change is observed until the temperature is sufficiently high to cause a decrease. With a maximal stimulus and when the temperature of the stretches *a* and *c* was not far from room temperature this has been my uniform experience. If we call the

temperature which the whole nerve has before the changes in the temperature of the stretch *b* begin, the " base-line temperature," we may state that from a base-line temperature between 15° and 25° there is no increase in the action current when the nerve impulse has to pass through a heated area, provided a strong stimulus has been used.

If the base-line temperature be as low as 10°, there may be an increase in the action current, as is shown by the following curve (Fig. 5).

Two other experiments indicate the same conclusion. It may be that cooling the whole nerve down to 10°, or lower, so heightens its irritability that it gives a greater action current when the impulse passes through a heated area. Boruttau[1] has called

FIGURE 5. — From an experiment of Jan. 30. Kept nerve; demarcation current at beginning of experiment = 61 mm.; demarcation current at end of experiment = 44 mm. See Fig. 4 for explanation.

attention to the beneficial effect of a moderately low temperature for the action current. He found that warming the entire nerve caused a decrease in the action current. " There exists therefore a temperature optimum, which in the case of the frog is quite low." Some of my own experiments point to the same result. Perhaps had the base-line temperature in my experiments been 10° C. or lower, the results with a strong stimulus might have been different.

I should therefore emphasize the necessity of giving the temperature of all parts of the nerve in such experiments as those under

[1] BORUTTAU : Centralblatt für Physiologie, 1898, xii, p. 317.

consideration, in order to qualify them properly. This precaution is quite important, as there exists a seeming contradiction in published results which depends upon this fact. Thus Gotch and Macdonald,[1] contrary to Boruttau as above quoted, state: "There is therefore no doubt that nerve is more readily excited by such stimuli as break induced currents when warmed: and similar observations with the make induction currents showed the same favourable effect of warmth." This result of Gotch and Macdonald's refers to the case in which only the stimulated point, not the whole nerve, is warmed. Boruttau's statement refers to the temperature of the entire nerve, and falls into line with the observations upon cooled frogs, the heightened irritability of whose nerves is well recognized.

If we designate by weak stimulus a stimulus calling forth but a small fraction of the maximal galvanometric response, and by moderate stimulus one between this strength and the maximum, we may apply what has been said about the response to the strong stimulus to the response following a moderate stimulus, *i. e.* there is no increase in the action current. So far the results agree with those obtained in the spring of 1899. With a weak stimulus, however, I have observed a very few cases of an increase. When the increase was most pronounced the action current that showed it was but a small fraction of the maximal response, $\frac{1}{5}$ to $\frac{2}{10}$. These few positive results are to be found among the reported experiments, Jan. 9, Jan. 11, Jan. 27, and Feb. 2 (pages 326–331).

On the other hand, fifteen experiments in which the response was one-third of the maximal and less, show no increase. The results are therefore in the main negative. Out of sixty-seven experiments which make up this division, and in which all strengths of stimulus were employed, from minimal to maximal, there are only three or four which are quite positive in showing an increase in the action current when the nerve impulse passes through a warmed area. We are therefore justified in saying that the increase which the nerve impulse suffers upon passing through a heated stretch is difficult to demonstrate with the galvanometer.

I call especial attention to the experiment under date of Jan. 9 (a part of which is here plotted in the form of a curve, see Fig. 6 B), in which repeatedly with a rise of temperature of the stretch *b* the action current increases. It is also worthy of note that in this exper-

[1] GOTCH and MACDONALD: Journal of physiology, 1896. xx, p. 270.

iment the results obtained with a strong stimulus (Fig. 6 A) are clearly contrasted with those obtained with a weak stimulus. With the strong stimulus the action current decreases when the high temperature is reached; with the weak stimulus it is increased; the other experiments show the same phenomenon.
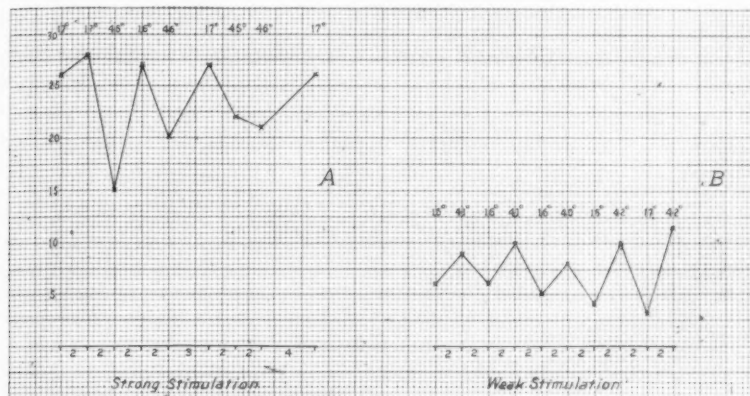
FIGURE 6. — From experiment of Jan. 9. A plotted from observations 38–46 (inclusive); B plotted from observations 55–64 (inclusive). In A secondary coil distance = 100 mm.; in B secondary coil distance = 143 mm. Ordinates express strength of action current; two ruled divisions are equal to 1 mm. of scale deflection; temperature of stretch *b* given above; intervals between observations, in minutes, along abscissa.

The result of these experiments at first sight does not speak for the view that the action current may be taken as a true measure of physiological activity; for it is very easy to show that passing the nerve impulse through a heated area of nerve will increase a minimal muscle contraction to a maximal. This fact has already been mentioned, and I have repeatedly verified it. With the galvanometer the increase seems to be very difficult to obtain with any but a weak stimulus, and is by no means a constant phenomenon even under this condition.

Some light may be thrown upon this difference between the effects of strong and very weak stimulation if we consider the curve which expresses the relationship between the strength of stimulus and the resulting action current (Waller,[1] Greene[2]). In this curve (Fig. 7)

[1] WALLER: Brain, 1895, xviii. p. 213.
[2] GREENE: This journal, 1898, i. p. 104.

we are struck by the great extent which lies wholly out of the range
of functional impulses. The strength of stimulus necessary to call
forth a maximal muscle contraction lies low down on the curve. If
we assume that the nerve impulse, when it passes through a region of
higher temperature, is affected only within the range of functional
activity, the results obtained with the galvanometer may in part be
explained. Leaving aside the cases of a low base-line temperature
and considering only those in which the base-line temperature lay
between $15°-25°$ C., it will be remembered that only exceptionally
was any increase in the action current observed. But if the above
assumption be correct, we should expect no increase unless the action
current represented a functional impulse, *i. e.* an impulse of the
dimensions normally occurring in the nerve. With the kept nerve
the action current is considerably greater than that of the fresh nerve,
and in such cases a small fraction of the maximal action current may
represent, so to speak, a normal impulse — one of the same intensity
as that conducted along a nerve when the muscle is thrown into
activity.

I performed a few experiments to determine the relative sensibil-
ities of the galvanometric and muscular responses to the nerve
impulse, and found that, using tetanic stimulation, the muscle was
called into activity with the secondary coil 200 mm. distant, such a
stimulus giving no action current; at 150 mm. the response was full
tetanus, while at this distance the action current was but a few
millimetres (*cf.* Steinach,[1] Boruttau,[2] for similar results also Bieder-
mann,[3] Waller,[4] for opposite). The nerve is capable of showing an
increase in the galvanometric response with increasing stimuli, far
beyond that given by the muscle; the muscle has reached its maxi-
mal response when the nerve, as it were, has just begun.

Within the range of functional activity there can be little
question from the effect upon the muscular contractions as to the
increase which the nerve impulse suffers when it passes through an
area of higher temperature. This is made most highly probable *a
priori* from the fact that, under favorable conditions, warming a nerve
may call forth a tetanic contraction. If heat can thus act as a
stimulus, we should expect that a portion of nerve, the temperature of

[1] Steinach: Archiv für die gesammte Physiologie, 1894, lv, p. 487.
[2] Boruttau: Archiv für die gesammte Physiologie, 1897, lxv, p. 1.
[3] Biedermann: Elektrophysiologie, 1895, p. 660.
[4] Waller: Brain, 1895, xviii, p. 213.

which had been raised, would be more responsive to an inflowing stimulus from the neighboring cooler portion. But if, beyond the functional range, the nervous response is governed by a different law of disassimilation (in Hering's sense[1]) then an increase in temperature may have no effect until it approaches the lethal limit.

Waller has shown, and Greene has confirmed his results with mammalian nerve, that the beginning of the energy curve for nerve is at first convex to the abscissa, followed by a long straight portion; this straight ascending limb is succeeded, as shown by Greene, by a straight horizontal one almost parallel to the abscissa.

The accompanying curve (see Fig. 7) is modified from Greene's. The dotted portion is greatly magnified with respect to the rest of the curve and is consequently made to extend beyond the origin of co-ordinates. The units are minute fractions of an ampère, and the unit of the action current is about $\frac{1}{1500}$ that of the stimulating current. Greene places the point of maximal muscular response well
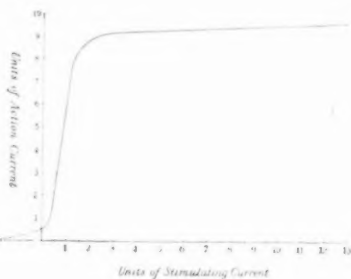
FIGURE 7.

up on the straight ascending portion, but I take it that this response was for single break shocks; for tetanic stimulation the point would lie considerably lower. If this initial convex portion and the beginning of the straight limb represent the range of functional activity, we see indeed that it is subject to a different law of disassimilation, and this fact may possibly account for the negative results obtained with stimuli beyond the functional range. Because we observe an increase in the muscular response when we allow the nerve impulse to pass through an area of warmed nerve, we are not perhaps justified in expecting the same result from the galvanometer, unless the instrument is capable of showing a deflection with a strength of stimulus which just calls the muscle into activity.

According to my experiments there is no doubt that from a baseline temperature of 15°–25° C. there is no increase in the action current when the impulse has to pass through a heated area, if the stimulus lies above what I have designated as a weak one, that is, one probably within the normal functional range.

[1] HERING: Lotos, 1889, N. F. ix, p. 36 (translation, Brain, 1897).

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STIMULATION BY CONDENSER DISCHARGE.

In these experiments the apparatus, with the exception of that part of it which delivered the stimulus, differed very little from the apparatus of the preceding division. The ∩-shaped glass tube was employed instead of the tunnel. In order to avoid polarization, two cells were used in connection with the condenser; they were so arranged that they charged the condenser first + —, then — +. To secure a tetanic stimulation the Bernstein rheotome was modified in the following way. An ebonite disc was brought below the revolving wheel of the rheotome; in this disc were sixteen brass plugs; alternate brass plugs put the two poles of the condenser into connection through the nerve; the intervening plugs charged the condenser, and they were so connected with the terminals of the cells that the discharge reversed itself at each discharging plug. The revolving wheel carried a platinum wire bent into a spring which swept over the plugs, thus establishing an alternating contact. The arrangement is made clear by the accompanying schema (Fig. 8.)
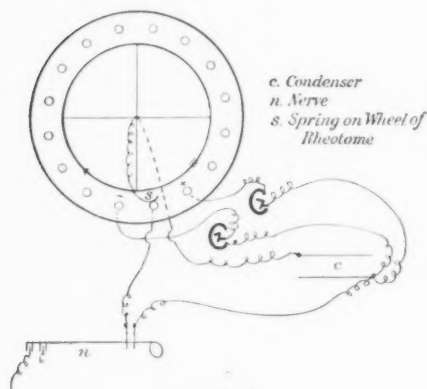
c. Condenser
n. Nerve
s. Spring on Wheel of Rheotome

FIGURE 8.

Each revolution of the wheel thus caused eight discharges through the nerve, each discharge being opposite to that which just preceded. The rheotome was driven by the Helmholtz motor, the movable coils of which were fed by two storage cells in parallel, the fixed coils being connected with one Edison-Lalande cell; thus supplied, with the governor screwed up, the motor ran well and constantly.

Not many experiments were done with this form of stimulus because of the difficulty of obtaining frogs of good size just at the time when these particular experiments were undertaken. They number, however, six in all.

From the experiment under date of Feb. 16 (Table III, page 332) it is seen that the effect of cooling the stretch *b* nearly to zero is nil, the action current seeming to suffer no decrease whatsoever. This was the general result of these experiments when the response to the series of condenser discharges was considerably less than the maximal response obtained by stimulating with induced currents. On the other hand, when the action current from the condenser discharge fell but little below that called forth by a secondary coil distance of 100 mm., there was a decrease in the action current, if the temperature of the stretch *b* fell to near zero. In order to determine whether a weak stimulus would also be little affected by low temperatures when faradic stimulation was used, I performed the following experiment (Table IV, page 333) which shows very distinctly that the action current resulting from the weak stimulus is little, if at all, affected.

When the temperature of the stretch *b* was raised, no increase of the action current from condenser discharges was observed, but uniformly a decrease as the temperature approached 40° C. In these experiments the nerve lay across a thin walled glass tube so that the temperatures were not so high as when the tunnel was employed.

It would seem, therefore, from these half-dozen experiments that galvanometric response is effected in the same way, whether the stimulus be started by condenser discharge or induced currents, when the nerve impulse passes through a cooled or heated area, except that no increase of the action current following weak stimulus was noticed with the condenser discharges. But in these experiments no such small fraction of the maximal action current was used as in the previous division.

REFLEX STIMULATION.

Since the few well marked cases of increase in the action current had been observed when there was only a small fraction of the maximal galvanometric effect, Professor Howell suggested that I try to get a negative variation upon reflex stimulation, and then to interpose a warming tube between the sciatic plexus and the non-polarizable electrodes which led off to the galvanometer. With such an arrangement we should expect the action current, due to a normal nerve impulse, to show an increase.

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About a dozen experiments were performed, but only three or four gave any results at all, and these were negative as far as any increase in the action current was concerned. The first frogs used were of medium size, and were kept in the cellar of the laboratory. They were either curarized or had their brains destroyed; in the latter case they were pinned out upon a cork board to prevent any movement of the nerve on the electrodes. No negative variation could be obtained from reflex stimulation in this first set of experiments.

In a new lot of frogs received in the laboratory about the first of April were five which were very large and vigorous. They were cooled after the manner recommended by Steinach[1] in order to get highly irritable specimens. The frogs were allowed to remain in the ice-chest for a week before using, and the day before the experiment they were placed directly upon the ice. Three of these were curarized, two were etherized during the preparation of the sciatics and then allowed to come out of the anæsthesia.

In the first experiment performed the skin over the thigh was stimulated by induction shocks; in the remaining experiments one sciatic was stimulated, while the other was connected with the galvanometer. An action current was observed in all but the first experiment; it was small, only a fraction of a millimetre in most instances, though in one experiment a deflection of as much as 9 mm. was observed, followed by a complete return of the image to its initial position. In this last experiment, however, the frog was not curarized, and hence this considerable deflection might have been due to some movement of the nerve upon the electrodes; for it was difficult to tie the frog down so securely as to prevent all movement.

VI. Conclusions.

The main results obtained in this investigation may be briefly summarized as follows : —

1. When the stimulus used is an induction current or a condenser discharge sufficient to cause a strong or moderate action current, this current is not changed in intensity by causing the nerve impulse to pass through areas of different temperatures within a range lying between 8° or 10° C. on the one side and 35° to 40° C. on the other,

[1] STEINACH : Archiv für die gesammte Physiologie, 1896. lxiii. p. 495.

provided that the remainder of the nerve including the stimulated and led-off portions is kept at a temperature above 10° C.

2. A similar result was obtained with the feeble action currents produced by reflex stimulation.

3. Under the conditions specified in (1) the action current shows usually a progressive decrease in extent when the nerve impulse is passed through an area lower in temperature than 8° C. or higher than 40°–42° C. Complete disappearance of the action current was observed at —7° C. and 47° C.

4. With very weak induction currents or condenser discharges the action current shows no decrease in passing through an area whose temperature is close to zero.

5. A distinct increase in the strength of the action current as a result of passing the nerve impulse through a warmer area (between 10° C. and 40° C.) may be obtained with favorable nerves under two conditions : (*a*) when the stimulus is sufficiently weak to cause an action current of only $\frac{1}{9}$ to $\frac{1}{20}$ of the maximal action current; (*b*) when the entire nerve with the exception of the portion warmed is kept at a temperature below 10° C. An increase in the nerve impulse under similar conditions may be demonstrated much more easily when the muscular response, instead of the action current, is taken as an indicator.

6. The action current ordinarily observed and studied probably lies far beyond those accompanying maximal functional impulses, and, though physiological, is produced only upon direct stimulation of the nerve.

It is a pleasant duty to acknowledge my indebtedness to Professor Howell, and to thank him for his kindly advice and encouragement given during the progress of this investigation.

TABLE I.

Effect upon the action current caused by passing the nerve impulse through areas of high or low temperatures. The time of beginning is given, and thereafter the intervals between the observations in minutes.

Experiment No. 26. Nerve kept 24 hrs.; threaded through tunnel.					
No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
1	57	8.2	—	—	
2	59	11.0	—	—	
3	61	11.5	—	—	
4	59	9.5	25°	h. m. 10 41	Stimulus made weaker
5	—	—	45°	1½	Temperature of moist chamber, 25° C.
6	58	0.5	48°	1	
7	—	—	23°	½	
8	59	8.5	23°	1½	
9	58	8.5	25°	3	
10	55	8.2	25°	3	
11	—	—	45°	1	
12	—	8.2	47°	½	
13	—	3.8	49°	1	
14	—	1.5	—	¾	
15	—	0.7	—	¼	
16	—	0.1	—	½	
17	—	0.0	48°	½	
18	—	5.1	24°	1	
19	53	5.2	23°	4	Temp. of moist chamber, 26°

TABLE I (*continued*).

	Experiment No. 28. Nerve kept 24 hrs.; threaded through tunnel.				
No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
1	53	11.0	—	—	Temperature of moist chamber, 23°–26° C.
2	51	11.3	—	h. m. 11 27½	
3	—	—	–5°	½	
4	—	4.5	—	¾	
5	—	3.2	–6°	½	
6	—	1.8	–6°	½	
7	—	1.0	—	½	
8	—	0.0	—	¾	
9	—	11.5	23°	¼	
14	29	8.7	—	12 11	
15	29	8.8	24°	3	
16	—	—	38°	1	
17	30	8.1	39°	1¼	
18	31	8.2	39°	2¼	
19	—	—	24°	¼	
20	—	8.3	24°	1¼	
21	31	8.7	24°	2½	
22	—	—	4°	1	
23	28	7.9	3°	2¼	
27	20	5.0	3°	7	
28	20	9.8	24°	1¼	

TABLE II.

Effect upon the action current caused by passing the nerve impulse through areas of high and low temperatures with varying strengths of stimuli (induced current). The time of beginning is given, and thereafter the intervals between the observations in minutes.

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch b.	Time.	Remarks.
				Jan. 9, A.M., 1900. Nerve kept; scissor section; non-polarizable electrodes quite isoelectric. S. C. = distance of secondary coil.	
				h. m.	
1	103	23.3	14°	11 53	S. C. 100 mm.
9	—	24	16°	12 10	S. C. 100 mm.
10	—	25.2	40°(?)	2	2 min. interval.
11	—	26.5	15°	2	
12	—	24.2	45°	2	
13	—	26.3	12°	2	Experiment discontinued until P. M.
1	138	25.2	—	2 56½	Fresh section. S. C. 100 mm.
6	102	3.3	16°	3 15	S. C. 150 mm.
7	—	2.8	17°	2	
8	—	2.7	18°	2	
9	—	3.6	21°	2	
10	—	2.3	16°	2	
11	—	4.0	23°	2	
12	—	3.6	18°	3	
13	79	4.3	28°	3	
14	—	4.0	31°	2½	
15	—	4.3	34°	2½	
16	—	3.6	36°	2	
17	—	4.3	38°	2	
18	—	5.0	40°	2	
19	—	3.6	42°	2	

TABLE II (*continued*).

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
20	—	4.5	45°	2	
21	—	3.0	18°	2	
22	—	2.5	17°	2	
23	66	2.0	17°	2½	
24	—	3.8	41°	2½	
25	—	3.0	41°	2	
26	—	2.5	17°	2	
27	—	1.9	16°	2	
28	60	2.7	16°	2	
29	—	1.4	16°	2	
30	—	4.2	40°	2	
31	—	1.5	16°	2	
32	—	4.0	42°	2	
33	—	1.5	16°	2	
34	—	?	40°	2	
38	55	26.1	17°	8	S. C. 100 mm.
39	—	28.3	17°	2	
40	—	15.3	45°	2	
41	—	27.3	16°	2	
42	—	20.0	46°	2	S. C. 100 mm.
43	—	27.0	17°	3	
44	—	22.3	45°	2	
45	—	21.2	46°	2	
46	48	25.8	17°	4	From 6–46, tank supply 16°–19° C.
52	—	11.8	16°	12	S. C. 140 mm.
53	—	6.2	16°	2	S. C. 143 mm.
54	—	7.7	41°	2	

TABLE II (*continued*).

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
55	39	6.0	16°	2	
56	—	8.6	41°	2	
57	—	6.0	16°	2	
58	—	10.0	41°	2	
59	—	5.0	16°	2	
60	—	7.9	40°	2	
61	—	3.7	16°	2	
62	—	9.6	42°	2	
63	—	3.3	17°	2	
64	—	9.0	42°	2	
65	—	3.3	16°	2	
66	—	10.0	44°	2½	
67	37	3.8(?)	12°	2½	
68	—	2.9	17°	2	
69	—	8.9	45°	2	
70	—	3.0	16°	2	
71	31	24.3	16°	2	S. C. 100 mm.
72	—	17.5	46°	2	
73	—	28.0	18°	2	
74	—	21.0	45°(?)	2	From 52–74, tank supply 16°–17° C. At the end of the experiment the nerve was stiff between the tunnel and the leading-off electrodes.

TABLE II (*continued*).

JAN. 11, P. M., 1900.   Nerve kept; scissor section; non-polarizable electrodes 2.5 mm.[1]

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
1	55	17(?)	15°	h. m. 4 53	S. C. shoved over primary.
2	53	3.3	15°	2	S. C. 160 mm.
3	52	2.0	15°	2	
4	49	3.6	45°	3	
5	—	4.0	46°	1	
6	—	2.0(?)	18°	1½	
7	—	1.7	18°	2½	
8	—	3.6	45°	2	
9	41	2.9	46°	2	
10	—	2.4(?)	17°	2	
11	—	3.3	45°	2	
12	—	8.0	17°	3(?)	S. C. over primary.
13	—	0.0	50°	2	
14	—	2.0	17°	1	
15	—	3.5	17°	½	
16	—	0.0	48°	1½	
17	—	0.0	17°	2	From 1–17, tank supply 16°–19°. Nerve at the end of the experiment was in no way dried.

JAN. 27, 1900.   Nerve kept, end cooked by bringing it in contact with hot test-tube. Non-polarizable electrodes 1.5 mm.[1]

1	79	25.7	—	h. m. 10 17	S. C. over primary.
2	—	0.5	18°	7	S. C. 180 mm.
3	—	0.5	43°	2	
4	—	0.2	18°	2	1–4, tank supply 18° C.

[1] That is, gave a deflection of 2.5 mm. upon being joined by a short strip of filter paper moistened with physiological salt solution.

TABLE II (*continued*).

No. of observa-tion.	Demar-cation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
19	—	8.7	10°	11 0	S. C. 150 mm.
20	—	8.5	10°	2	
21	—	3.0	10°	1	S. C. 155 mm.
22	—	3.3	10°	1	
23	—	9.5	10°	1	S. C. 150 mm.
24	—	2.1	10°	1	S. C. 155 mm.
25	—	3.2	37°	2	
26	—	2.7	10°	2	
27	—	—	38°	1	
28	—	7.0	43°	$\frac{1}{2}$	
29	—	2.0	11°	$1\frac{1}{2}$	
30	—	—	40°	1	
31	—	7.0	45°	$\frac{1}{2}$(?)	
32	—	3.4	11°	$1\frac{1}{2}$	
33	—	—	38°	1	
34	—	5.2	42°	1	
35	60	4.2	11°	2	
36	—	—	38°	1	
37	—	6.3	44°	$\frac{3}{4}$	
38	—	3.3	11°	$1\frac{1}{4}$	
39	—	4.8	11°	1	
40	—	2.5	11°	1	
41	—	6.0	10°	1	
42	—	3.0	9°	1	19–42, tank supply 9°–11° C.
				11 27	

    The experiment was continued until at the end the nerve had dried, but if obser-vation 21 is compared with 42, the response of the nerve to the same stimulus is seen to remain unaltered.

    In this experiment instead of the tunnel, a ∩-shaped glass tube was used; across this lay the nerve.

TABLE II (*continued*).

Feb. 2, P. M.   Nerve kept; scissor section.   Non-polarizable electrodes 60 mm.; nerve lay across glass tube.

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
1	66	15	21°	3 35	S. C. 100 mm.
4	—	19.2	21°	6	S. C. 100 mm.
5	—	16.8	41°	2	S. C. 100 mm.
6	—	15.0	42°	1	
7	—	20.3	21°	1	
8	—	6.7	21°	1	S. C. 150 mm.
9	—	6.7	21°	1	S. C. 150 mm.
10	—	1.0	21°	1	S. C. 155 mm.
11	—	1.9	20°	2	S. C. 155 mm.
12	—	5.2	40°	2	
13	—	3.5	20°	1½	
14	48	4.7	20°	2	
15	—	1.0	19°	1	S. C. 157 mm.
16	—	1.1	19°	½	S. C. 157 mm.
17	—	5.9	41°	2	S. C. 157 mm.
18	—	1.3	19°	1	
19	—	3.0	18°	1	
20	—	5.2	38°	2	4-24, tank supply 18°-21° C.
21	—	5.3	39°	1	
22	—	1.0	18°	1	
23	—	5.2	41°	2	
24	—	1.1	18°	2½	Experiment was continued; at end nerve was somewhat stiff.

TABLE III.

Effect upon the action current caused by passing the nerve impulse through areas of high or low temperatures, when the stimulus used was condenser discharges. The time of beginning is given, and thereafter the intervals between the observations in minutes.

Feb. 16, P.M., 1900. Nerve kept (?); two Edison-Lalande cells charged the condenser one + --, the other -- +. Voltage of each cell, as measured by Weston voltmeter, = ¼ volt. Capacity of condenser = 0.5 microfarad.

No. of observa-tion.	Demar-cation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
1	77	4.1	21°	4 41	
2	—	5.3	23°	2	
3	—	5.3	22°	2	
4	—	5.3	2°	2	
5	—	6.0	2°	2	
6	—	6.5	2°	1	
7	—	6.0	2°	2	
8	—	6.7	2°	2	
9	—	6.5	24°	2	
10	—	6.8	24°	1	
11	76	5.7	24°	3	
12	—	5.6	25°	2	
13	—	5.9	4°	2	
14	—	6.0	5°	2	
15	—	6.0	7°	2	
16	—	6.0	20°	2	
17	—	6.0	20°	2	
18	—	5.7	34°	1½	
19	—	5.8	35°	1	
20	—	6.0	19°	1½	
21	—	5.9	19°	4	
22	—	5.3	40°	1½	
23	—	5.7	43°	1	

TABLE III (*continued*).

No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
24	—	6.0	19°	1½	
25	—	5.8	42°	1½	
26	—	4.7	45°	1	
27	—	6.3	20°(?)	1	2-27, tank supply 23°–26° C. for 1½ min. at 22, 28° C.
28	74	15.5	—	5½	Here change was made to induced current. S. C. = 100 mm.
29	—	21.5	—	1	
30	—	12.5	1°	1½	
31	—	11.8	1°	1½	Nerve at end of experiment in good condition.

TABLE IV.

Effect upon the action current caused by passing the nerve impulse through an area of very low temperature when the stimulus (induced current) is weak. The intervals between the observations are given in minutes.

Feb. 16, A. M. Nerve kept; thermal section. Stretch *b* lay over glass tube.					
No. of observation.	Demarcation current.	Action current.	Temp. (C.) of stretch *b*.	Time.	Remarks.
				h. m.	
26	—	15.2	17°	—	S. C. 120 mm.
34	—	3.3	17°	—	S. C. 160 mm.
35	—	4.0	17°	1	
36	—	3.5	17°	1	
37	—	4.9	17°	1	
38	—	5.5	2°	1	
39	—	4.0	2°	1	
40	—	6.0	2°	1	
41	—	4.3	2°	1	
42	—	5.9	2°	1	Tank supply 22°–26° C.

EXPERIMENTS CONCERNING THE PROLONGED INHIBITION SAID TO FOLLOW INJURY OF THE SPINAL CORD.[1]

By W. T. PORTER and W. MUHLBERG.

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IT is a well known fact that the excessive stimulation of afferent fibres may suspend the action of distant parts by inhibiting the nerve cells by which these parts are innervated. Such inhibition might continue hours, or even days, and of late years this possibility has greatly influenced students of the central nervous system. Believers in the automatic function of the spinal cord have taken refuge behind this hypothesis of prolonged inhibition, and it has not been easy to dislodge them. The strength of their position is made clear by the following considerations. In the highest vertebrates many important functions requiring the coördinated action of numerous muscles are accomplished through master cells in the brain acting upon subsidiary cells in the spinal cord and bulb. The respiratory mechanism is an example. The several sets of respiratory muscles are innervated by motor cells in the spinal cord and bulb. In contact with the nerve cells of the respiratory muscles lie neurons which have come from certain cranial cells termed collectively the respiratory centre. Periodic impulses descend from the respiratory centre to the spinal respiratory nerve cells and cause them to discharge motor impulses to the respiratory muscles, which thereupon contract in carefully coördinated sequence. Believers in the automatism of the spinal cord assert that the spinal respiratory cells possess independent automatic power by virtue of which they themselves periodically discharge the respiratory impulse. Their discharge is not compelled by impulses reaching them from the master cells of the so-called respiratory centre. The obvious reply to this contention is that the diaphragmatic respiration ceases when the phrenic nuclei are separated from the respiratory centre by the

section of the spinal cord. From this apparently crushing observation the hypothesis of prolonged inhibition offers a ready escape. The section of the spinal cord, it is urged, inhibits the phrenic cells for many hours. Life cannot be preserved by artificial respiration long enough for the inhibition to pass away. Were it not for this prolonged inhibition the phrenic cells would still carry on the respiratory movement of the diaphragm after their separation from the brain. The disordered contractions of the diaphragm observed upon interrupting the artificial respiration in animals in which the phrenic nuclei have been separated from the brain are not simply the reflexes easily provoked when the reflex irritability of the cord is raised by long-continued artificial respiration, but are caused by the automatic discharges from phrenic cells still partially inhibited. Similar use of the hypothesis of prolonged inhibition is made in the case of other functions.

Experiments disproving this hypothesis were published by W. T. Porter in 1895.[1] A transverse section of one side of the spinal cord was made between the phrenic nuclei and the brain. On severing the lateral tracts the diaphragm on the operated side became motionless. The advocates of spinal respiration would say that the phrenic cells on that side were inhibited. Nevertheless, when the phrenic nerve on the side opposite to the hemisection was severed, the diaphragm on the hemisected side instantly began to contract with a regular respiratory rhythm, in other words the phrenic cells on the hemisected side at once began to discharge rhythmical motor impulses. These cells therefore could not have been inhibited. Their failure to send out impulses after the hemisection must then have been due to the interruption of the habitual supply of respiratory impulses from the brain. On cutting the opposite phrenic nerve the respiratory impulses pursuing on the uninjured side of the cord their accustomed path from the brain to the phrenic nuclei were turned from their habitual way and entered the phrenic cells on the hemisected side. If, as has just been shown, the section of either half of the cord fails to inhibit the phrenic cells of that side, the section of both halves will not inhibit the cells of both sides. Consequently, the arrest of respiration which always follows the section of both sides of the cord cannot be due to inhibition. The arrest must then be due to the interruption of the respiratory impulses descending from the brain.

[1] PORTER, W. T.: Journal of physiology, 1895. xvii, pp. 455-485.

Hence the phrenic cells are not able to discharge automatic rhythmic respiratory impulses.[1]

The hypothesis of spinal respiration is so discredited by these and other adverse observations that further experiments in its disproof would hardly be necessary. But the hypothesis of prolonged inhibition is a far more important matter. Unchecked, this hypothesis would lead to the gravest misconceptions of the physiology and pathology of the central nervous system. Additional evidence therefore cannot fail to be welcome.

The purpose of the present investigation, then, is the long-continued observation of animals in which spinal cells known to take part in some automatic function have been separated from the brain, in order to determine whether the loss of function following the isolation is permanent or temporary. If permanent, the separated cells cannot be automatic in their action but must depend for their rhythmic discharge on stimuli received from other nerve cells. If the loss of function be only temporary, the isolated cells may have been inhibited or may have gradually developed powers disused since fœtal days.
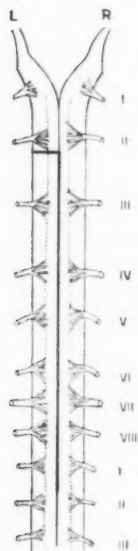
The phrenic cells were selected for the experiments. The two sets of phrenic nuclei were separated from each other by a median longitudinal section of the spinal cord. At the anterior (cranial) end of this median section, the cord was hemisected. The phrenic cells of one side were thus completely isolated from those of the opposite side and from the brain. (Fig. 1.) The animals were kept alive, and after days, or weeks, the isolated cells were shown to be still functional by making them discharge motor impulses, and the diaphragm was exposed and directly inspected to

FIGURE 1.— Diagram showing the isolation of the phrenic cells of one side from those of the opposite side and from the brain.

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[1] My friend Professor Jacques Loeb, in discussing these experiments not long ago, said to me that it would be interesting to keep alive for a number of days animals in which the spinal cord had been severed on one side between the bulb and the phrenic nuclei, as just described. Possibly the phrenic cells might after all discharge respiratory impulses automatically, if time enough were allowed for complete recovery from the operation. The experiments as published in the paper

determine whether the half of the muscle innervated through the isolated phrenic cells contracted rhythmically.

The animals employed were cats and rabbits. In the later experiments only rabbits were used, as the bleeding from the operation is less severe in these animals. The bleeding was lessened by feeding the rabbits on dry food. The instruments were always carefully sterilized. The skin in the field of operation was thoroughly wet in a solution of corrosive sublimate (1 : 2000) and the parts not so treated were covered with towels wrung out in the solution. The animals were well anæsthetized with a mixture of ether and alcohol (3 : 1). During the section of the spinal cord it is necessary that the anæsthesia be deep, as the slightest movement is fatal to success. The central portions of the laminæ of the vertebræ were removed usually from the II cervical to the I dorsal vetebra inclusive. The dura was divided in the median line. A longitudinal median section was then made with a cataract knife as a rule from the II cervical to the I dorsal vertebra inclusive, completely dividing one half of the cord from the other. The breathing was then carefully observed in order to determine whether both sides of the diaphragm contracted equally. In case one half seemed to contract more strongly than the other, the hemisection was made on the stronger side. The hemisection was always placed at the anterior end of the longitudinal incision. The lower margin of the cord at the point of division was lifted slightly to make sure that the half of the cord was completely severed. It is not easy to sever completely the lateral tract, which contains the descending respiratory fibres. Thus in three animals the inspection of the diaphragm some time after the operation revealed normal contractions on both sides. In each case the autopsy showed that the hemisection was not complete. In the cat of November 11, the lateral and anterior columns escaped section; in the rabbit of November 18, the section stopped about one half millimetre from the lateral margin; and in the rabbit of November 22, only that part of the lateral column next the gray matter was cut. Occasionally the hemisection was deferred until the animal recovered from the shock of the primary operation. In a few cases, it was made with a redhot needle, to lessen the bleeding, but on the whole this method

on the path of the respiratory impulse from the bulb to the phrenic nuclei are to my mind conclusive, but the hypothesis of prolonged inhibition is of such importance that the additional experiments described in the present article seemed to be justified. — W. T. P.

proved of little or no advantage. The skin wound was now sewed up. No attempt was made to stitch the dura. The wound was covered with corrosive sublimate gauze over which was placed a thick pad of sterile absorbent cotton. The animal was put on a straw bed in a warm place, and covered with a thick light coverlet. The head was kept somewhat lowered to guard against anæmia of the brain. Beginning the second or third day the rabbits were fed some pieces of carrot.

The surgical shock following the operation continued often twenty-four hours and was especially marked in animals that had lost much blood. Such animals were injected subcutaneously on the second or third day with about 30 c.c. of 0.8 per cent sodium chloride solution. The skin and tendon reflexes were commonly suspended on the side of the hemisection immediately after the operation. Generally they reappeared within twenty-four hours. Later they became exaggerated. Usually the animals seemed very irritable after the second day. Suppuration did not occur in any case.

When the time came for the inspection of the diaphragm, the animal was anæsthetized and the abdomen opened so that the muscle could be observed directly. When one half of the diaphragm is passive, the artery at the margin of the central tendon is dragged towards the opposite side at each inspiration, the passive side is pale and does not become paler when it moves, the individual fibres do not shorten, and the anterior muscular slip on the passive side can be seen distinctly to rest while its fellow contracts. Efforts were made to cause the isolated cells to discharge motor impulses. For this purpose their irritability was increased by prolonged artificial respiration and by strychnine. Finally an autopsy was made and the cord, heart, and lungs were examined.

It has been pointed out in another place [1] that both sets of phrenic nuclei can be divided from each other by a median longitudinal incision without inhibiting respiration. The same observation was repeatedly made in the present investigation. In no case were normal contractions suspended longer than ten or fifteen seconds. In two apparent exceptions one side of the diaphragm ceased contracting. But the autopsies showed that in one (November 8, 1899) the incision deviated from the median line, and in the other (November 13, 1899) a large clot formed under one side of the cord. The

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[1] PORTER, W. T.: Journal of physiology, 1895, xvii, p. 462.

failure of this severe mechanical stimulus of the phrenic cells to cause inhibition will be especially significant when the reader is reminded that the two sets of phrenic cells communicate with each other by means of nerve paths. The longitudinal section must sever all these communicating paths and the gross mechanical stimulus must by them be conveyed directly into the cells. The changes which follow the operation also fail to check respiration. It might be supposed that the continued irritation produced by this wound so near the phrenic cells would inhibit their action. This, however, is not the case. Thus, on December 14, 1899, the cord in a rabbit was divided longitudinally and five days later direct inspection of the exposed diaphragm showed both sides contracting normally. At the autopsy a complete longitudinal section was found extending from the third cervical to the second dorsal vertebra. In another rabbit, the longitudinal section was made January 20, 1900, and six months after the operation the diaphragm was still contracting normally.

The experiments in which the phrenic nuclei of one side were completely separated from the brain by longitudinal section followed by hemisection are recorded in Table I.

It will be observed that animals were kept alive from one to twenty-four days after the separation of the phrenic nuclei of one side from the brain. *In every instance the separation of the phrenic cells from the brain caused the permanent arrest of the corresponding half of the diaphragm.*

This permanent arrest cannot be explained by a disturbance of the nutrition of the isolated cells. Proof that the respiration remains normal in spite of the long median section of the cord has already been given. It is not to be supposed that a transverse section above the phrenic nuclei could so impair their nutrition as to keep them functionless during the long periods of observation here recorded. In the paper on " The path of the respiratory impulse from the bulb to the phrenic nuclei " it was shown that five hours after hemisection the phrenic cells on the operated side were capable of taking part in the normal rhythmic respiratory discharge. The experiments of Wertheimer[1] and others, in which the whole cord was severed between the phrenic nuclei and the brain and life preserved for a short time by artificial respiration, also show that the phrenic cells

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[1] WERTHEIMER, E.: Journal de l'anatomie et de la physiologie, 1886, pp. 458–507.

TABLE 1.

List of experiments in which the phrenic nuclei of one side were isolated from the brain and the animal kept alive to determine whether the corresponding half of the diaphragm would resume its contractions.

Animal.	Phrenic nuclei isolated on one side.	Abdomen opened for inspection of diaphragm.	Interval between isolation of phrenic cells and inspection of diaphragm.	Position of sections shown post mortem.	
				Longitudinal section.	Hemisection.
	1899	1899			
Cat	Nov. 10, 8.30 A.M.	Nov. 11, 5 P.M.	32 hours	IV to VII cervical	Left, IV cervical
Cat	Nov. 14, 8.30 A.M.	Nov. 15, 8.30 A.M.	24 hours	IV cervical to II dorsal	Left, IV cervical
Rabbit	Nov. 17, 8.30 A.M.	Nov. 18, 11.30 A.M.	27 hours	II to VI cervical	Left, II cervical
Rabbit	Nov. 23, 8.30 A.M.	Nov. 24, 2.30 P.M.	30 hours	III cervical to II dorsal	Left, III cervical
Rabbit	Dec. 2, 8.30 A.M.	Dec. 5, 4.30 P.M.	3 days, 8 hours	IV cervical to II dorsal	Left, IV cervical
Rabbit	Dec. 4, 8.30 A.M.	Dec. 9, 2 P.M.	5 days, 5 hours	II cervical to II dorsal	Left, II cervical
Rabbit	Longitudinal section Dec. 14, 8.30 A.M. Hemisection Dec. 19, 9.30 A.M.	Dec. 21, 3 P.M.	2 days, 5 hours	III cervical to II dorsal	Left, III cervical
	1900	1900			
Rabbit	Longitudinal section Jan. 4, 9 A.M. Cross section	Jan. 6, 9.30 A.M.	11 days, 5 hours	II cervical to I dorsal	Left, II cervical
Rabbit	Jan. 8, 9 A.M.	Jan. 14, —	6 days	II cervical to II dorsal	Left, II cervical
Rabbit	Jan. 12, 9 A.M.	Jan. 15, 4 P.M.	3 days, 7 hours	I cervical to I dorsal	Left, II cervical
Rabbit	Jan. 23, 9 A.M.	Jan. 25, 4 P.M.	2 days, 7 hours	II cervical to I dorsal	Left, II cervical
Rabbit	Jan. 24, 9 A.M.	Jan. 29, 4 P.M.	5 days, 7 hours	II cervical to I dorsal	Right, II cervical
Rabbit	Feb. 17, 3 P.M.	Feb. 20, 3 P.M.	3 days	II cervical to I dorsal	Left, II cervical
Rabbit	March 12, 3 P.M.	April 5, 3 P.M.	24 days	II cervical to I dorsal	Left, II cervical
Rabbit	March 19, 3 P.M.	March 21, 4 P.M.	2 days, 1 hour	II cervical to I dorsal	Left, II cervical

remain functional after such a transverse section, for when the artificial respiration was suspended reflex contractions of the diaphragm were readily produced. Similar proof was sought and found in the present investigation. The isolated cells were made to discharge motor impulses.

In four animals (December 4, January 4, January 23, March 19) prolonged artificial respiration was employed. In the rabbit of January 4, the interruption of the artificial respiration was followed by five or six contractions of the diaphragm on the side of the hemisection. These contractions were spasmodic and were not synchronous with those of the normal side. The animal was very irritable.

In eight animals (November 17, 21, 23, December 2, 4, 14, January 4, 12) asphyxia was brought on by closing the trachea. In every instance irregular spasmodic contractions of the diaphragm on the side of the hemisection were observed when the asphyxia became extreme. Usually they did not appear until general convulsive movements began. They were not synchronous with the contractions on the uninjured side and did not at all resemble the normal respiratory movement. They disappeared when the phrenic nerve of that side was severed (December 4), so that they could not have originated in the diaphragm but must have been the result of impulses proceeding from the phrenic cells.

Strychnine was used in four rabbits (January 4, 24, February 17, March 19), and in every case it produced spasmodic contractions of the diaphragm on the side of the section. Like the spasmodic contractions in asphyxia, they were irregular, not followed by full relaxation, and not synchronous with the normal contractions. They did not appear as a rule until general convulsions began.

There is therefore abundant evidence that the arrest of respiration on the side on which the phrenic cells have been isolated is not caused by interference with the nutrition of the cells or by any other injury done them.

CONCLUSION.

When the phrenic cells of one side of the spinal cord are separated from the respiratory centre in the brain the rhythmic respiratory contractions of the corresponding half of the diaphragm are arrested. This arrest is held to be an excellent example of prolonged inhibition in consequence of injury to the spinal cord. It is shown in the

present investigation that the phrenic cells after their isolation are still able to discharge motor impulses, but that their apparent automatic rhythmic respiratory power is gone. This loss is permanent. It cannot be ascribed to inhibition. The collapse of the hypothesis in this conspicuous instance warrants the belief that injuries of the spinal cord do not cause prolonged inhibition.

SOME WAYS OF CAUSING MITOTIC DIVISION IN UNFERTILIZED ARBACIA EGGS.

By ALBERT P. MATHEWS.

[From the Marine Biological Laboratory, Woods Holl, Mass.]

HERTWIG [1] in 1895 first showed that it was possible to induce karyokinetic division of sea urchin eggs by exposing them to the action of strychnine sulphate. Morgan [2] in 1898 found that placing the eggs in sea water of higher osmotic pressure than normal inaugurated cell division when the eggs were returned to sea water. Mead [3] in 1898 showed that the eggs of Chaetopterus could be made to divide by placing them in sea water to which KCl had been added. Morgan [4] in 1899 confirmed Hertwig's observations as to the action of strychnine, and expressed the opinion that the eggs were in a state of unstable equilibrium and would react to various stimuli by division just as other cells reacted in other ways to these same stimuli. He compared the reaction to that occurring in the muscle cell. I believe a comparison to the nerve cell would have been better since these agencies (*e. g.* strychnine) affect the nerve cell more than the muscle cell. Loeb [5] in 1899, in his beautiful work on artificial parthenogenesis, found that exposure of Arbacia eggs for a very short interval to sea water to which alkali or acid had been added would start cell division. In 1894 the author [6] observed that unripe starfish eggs could be made to extrude the polar globules by vigorous shaking.

The essential nature of the change in the egg which sets up karyokinesis and produces the well known division figures, asters and spindles, has hardly as yet been brought in question. Loeb [7] in

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[1] HERTWIG: Sitzungsberichte. Gesellschaft für Morphologie und Physiologie, München, 1895.

[2] MORGAN: Archiv für Entwickelungsmechanik der Organismen, 1899, viii, p. 448.

[3] MEAD: Lectures, Woods Holl, Boston, 1898.

[4] MORGAN: Science, 1900, N. S., xi, p. 176.

[5] LOEB: This journal, 1899, iii, p. 447.

[6] MORGAN: Anatomische Anzeiger, 1894, ix, p. 150.

[7] LOEB: This journal, 1900, iv, p. 183.

a recent paper suggests that we are dealing with a liquefaction of certain elements in the egg, possibly of the nuclear membrane, which directly or indirectly results in cell division. In 1899 I suggested [1] that blood clotting and karyokinesis had apparently many points of similarity and that the processes were possibly identical. The experiments in the present paper indicate, I believe, that we are dealing rather with a process of liquefaction as Loeb suggests than with a process of clotting.

I have found that karyokinetic nuclear division followed by cell division may be produced by lack of oxygen, by heat, and by exposure to ether, alcohol, and chloroform.

Lack of Oxygen. — The Arbacia eggs were removed with all care to avoid contamination with sperm. They were placed in an Engelmann gas chamber in sea water and the oxygen expelled by hydrogen gas. The hydrogen was passed successively through sodium hydrate, potassium permanganate, sodium hydrate and water to remove all traces of acid. The gas was allowed to flow through the chamber in a lively stream for twenty to thirty minutes and then oxygen was allowed entrance for ten minutes and this followed by another exposure to hydrogen for twenty minutes. The eggs were then transferred to fresh sea water, and left exposed to the air.[2] After lying in the fresh sea water for from thirty minutes to two hours one or several large clear areas appear in the eggs resembling the clear areas (asters) in fertilized eggs, and division into two to eight cells takes place. This division may be fairly regular, but is more apt to be of the irregular type seen in eggs exposed to strong salt solutions. The cells do not live long, but as a rule disintegrate in the course of six to eight hours.

The exact behavior of the eggs varies greatly in different individuals. In some an exposure of fifteen minutes to hydrogen followed after an interval by a second fifteen-minute exposure will suffice to start them. In other cases a much longer exposure is necessary. Some eggs develop four to six clear areas and break up into several cells very soon after removal, while others develop large single clear areas in the middle of the egg only, in from two to three hours after removal from the gas chamber.

A continuous immersion in hydrogen never causes the appearance of the asters, but after a few hours most of the eggs die and many

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[1] MATHEWS: This journal, 1899, iii, p. 180.
[2] In covered dishes to prevent evaporation.

liquefy at once on exposure to oxygen, or even while remaining in the chamber. Oxygen appears to be necessary for developing the clear areas (asters).

By repeatedly interrupting the hydrogen gas and exposing the eggs to oxygen the total or partial liquefaction of the eggs is greatly facilitated. Eggs from the same female which preserved their form and color intact for hours in the hydrogen atmosphere would liquefy and dissolve quickly if exposed intermittently to oxygen. This has an interesting parallel in secretion in which the condition of vascular dilation and abundance of oxygen appears to hasten the decomposition of metabolic products in the cell stored up during a time of vasoconstriction or oxygen poverty.

Sections of the eggs after exposure to hydrogen gas show well defined division figures. The minute changes have not been carefully worked out, as this was outside the scope of the present paper, but it may be seen that the nucleus increases greatly in size and moves to the centre of the egg. It becomes surrounded by a hyaline material from the surface of which strong rays extend to the periphery of the cell, a stage corresponding to Wilson's caterpillar stage. The nuclear wall and the coarse rays disappear and centrosomes appear at three or four points near the nucleus, a multipolar spindle being formed of about the same size as the enlarged nucleus. Several small nuclei are formed and cell division of an irregular type follows.

Heat. — If the eggs are warmed to $32°–33°$ C. for two to four minutes and then returned to sea water many of the eggs develop clear areas and segment exactly as do the eggs exposed to hydrogen. Fewer eggs develop and death comes sooner. If the eggs are heated a little too long, four to five minutes, complete liquefaction of all eggs quickly follows. Heat, in other words, acts precisely as lack of oxygen in causing cell division and liquefaction.

Ether, Chloroform, and Alcohol. — Exposure of the eggs to a saturated solution of ether [1] in sea water for ten to fifteen minutes leads to the karyokinetic division of nearly all the eggs, the phenomena of division being the same as those in the asphyxiated eggs. Exposure to the sea water containing dissolved chloroform for three minutes produces a similar result, although more eggs disintegrate

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[1] That I should try the effects of ether on these eggs was suggested to me by Prof. E. B. Wilson, who had already observed its action on the astral rays of fertilized eggs.

and fewer divide. If the eggs are placed in sea-water, to each ten cubic centimetres of which one cubic centimetre of 50 per cent alcohol has been added, and left there for ten to fifteen minutes, they segment into several cells upon replacing them in sea-water.

CONCLUSIONS.

The foregoing experiments are of interest because they show that known methods for causing liquefaction in protoplasm will induce karyokinesis in these eggs. Among the agencies causing such liquefaction the hydroxyl ion is most powerful; this is followed by the hydrogen ion in small amounts, by lack of oxygen, by strychnine, quinine, pilocarpine and similar poisons, by loss of water from the cell, replacement of calcium or other ions by potassium, by ether, chloroform and alcohol and by a slight increase in temperature. If any of these agents are carried beyond a very narrow limit a complete liquefaction of the protoplasm results. Budgett[1] and Zoethout[2] have shown that the liquefaction of infusoria by poisons, high temperature and other means bears a remarkable resemblance to the action of lack of oxygen, and have suggested that these poisons are poisonous in that they interfere with the oxidations in the cell. It is an interesting question whether they interfere with respiration, thus leading to hydrolytic splittings and liquefaction, or whether they produce acid in the cell which in its turn interferes with oxidation. Probably the two processes mutually interact, the lack of oxygen leading to the production of acid, and the acid in its turn interfering with oxidation when oxygen is admitted, thus leading to the production of more acid. Very small quantities of the hydroxyl ion appear to be necessary to, or facilitate, oxidation in the cell as elsewhere in nature.

The liquefying action of loss of water by the cell may be seen very readily in the large colorless corpuscles of the Arbacia body fluid. These corpuscles swell, liquefy, and dissolve when brought into sea-water to which sodium, potassium, or magnesium chlorides have been added. This fact of the liquefying action of the loss of water by protoplasm has not been considered in dealing with the absorption of hypertonic solutions from the body cavities.

[1] BUDGETT: This journal, 1898, i, p. 98.
[2] ZOETHOUT: This journal, 1899, ii, p. 220.

The foregoing results indicate, I believe, that whatever the details of the process may prove to be, the essential basis of karyokinetic cell division is the production of localized areas of liquefaction in the protoplasm. The dissolution of the yolk and the enormous accumulation of hyaloplasm in the nucleus and centrospheres during karyokinesis clearly point in this direction. They certainly suggest that karyokinesis is accompanied by, if not due to, a process recalling a digestion of the cellular elements. The centrosome might be a liquefying enzyme.

Finally if such remarkably characteristic and definite structures as the asters with their rays are but the expression of currents in the cell protoplasm, as suggested by Bütschli,[1] Wilson,[2] and others, what shall we say for the other structures? I have already pointed out that in secreting cells, their well marked striation is probably due to the passage of fluid through the cell. Is it possible that the longitudinal striation of the axis cylinder process is due to the passage of the nerve impulses, or of other currents up or down the nerve?

[1] BÜTSCHLI: Archiv für Entwickelungsmechanik der Organismen, 1900, x. p. 52.

[2] WILSON: Lecture delivered at Woods Holl, August, 1900.

ON THE METHODS OF ESTIMATING THE FORCE OF VOLUNTARY MUSCULAR CONTRACTIONS AND ON FATIGUE.

By SHEPHERD IVORY FRANZ.

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CONTENTS.

I. METHODS OF ESTIMATING VOLUNTARY MUSCULAR ABILITY.

TWO general methods have been employed for the determination of the force of voluntary muscular contraction; in one the muscle contracts to its fullest extent, in the other method the muscle does not shorten, or shortens very little, and the energy is converted mainly into tension. The convenient terms *isotonic* and *isometric* are used respectively to designate these methods. Of the isotonic method there are two varieties: in one a weight is employed; in the other, a spring. In the isometric method a muscle contracts against the force of a stoutly resisting spring.

Isotonic Method. — *Weight ergograph.* The weight method, which was originally devised for extirpated muscles, seems to have been first applied to voluntary muscular contraction by Mosso[1] about 1884.[2]

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[1] Mosso: Archives italiennes de biologie, 1890, xiii. p. 123; Archiv für Physiologie, 1890. p. 89: La fatigue, translated from the Italian, 1894.

[2] Mosso's work did not appear until 1890, but some of his published records bear dates several years earlier.

In this method, it will be remembered, the muscle raises a weight, the height is noted to which the weight is lifted, and the mechanical work accomplished is calculated by the formula, *work = weight × height*. This weight ergograph has been employed by Mosso, by his co-workers,[1] and by many later investigators for the determination of the laws of fatigue. A series of contractions was made and the extent of movement was found to decrease gradually until no movement was noticeable. Muscular power was also estimated by adding the extent of the contractions and by calculating from this result the mechanical work done by the muscle. The effect of fatigue and of other conditions upon the accomplishment of work, and the gradual loss of power (the course of fatigue) were thus determined.

Spring ergograph or ergometer. The use of a spring instead of a weight for the determination of muscular power has been recommended by Cattell,[2] by Binet and Vaschide[3] and by many others.[4] Cattell has used both extension and compression springs. His extension spring was one that extended four millimetres for every kilogram weight added.[5]

With a muscle working against the force of a spring two measurements may be made; either the tension overcome may be determined by the application of the formula kd, in which k is the extension or compression constant of the spring and d is the distance of expansion of compression, or the mechanical work that the

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[1] MAGGIORA: Archives italiennes de biologie, 1890, xiii, p. 187; Archiv für Physiologie, 1890, p. 191, p. 342; LOMBARD: Archives italiennes de biologie, 1890, xiii, p. 371; American journal of psychology, 1890, iii, p. 24; Journal of physiology, 1892, xiii, p. 1.

[2] CATTELL: Science, N. S., 1897, v, p. 909; 1899, ix, p. 251; Psychological review, 1898, v, p. 151 ; 1899, vi, p. 159.

[3] BINET and VASCHIDE: Anné·psychologique, 1898, iv, pp. 245, 253, 303; BINET and HENRI: La fatigue intellectuelle, Paris, 1898. See especially pp. 178–180.

[4] FULLERTON and CATTELL (On the Perception of Small Differences. Published by the University of Pennsylvania, Philadelphia, 1892) used a spring to determine the accuracy of the force of movement. WELCH (This journal, 1898, i, p. 283) tested with a spring ergograph muscular power during mental activity. The details of the instrument are not given. SCRIPTURE has also devised a spring ergograph (Studies from the Yale psychological laboratory, 1897, iv, p. 69).

[5] The present writer has not seen the instrument devised by Binet and Vaschide, and their description is not sufficiently clear to enable any one to judge in what essentials it differs from the ergometer used by Cattell. In this paper, therefore, the spring ergograph of Cattell will be taken as a type of the isotonic spring ergographs and the criticisms will be based upon its use.

muscle has done may be calculated by means of the well known formula, representing Hooke's law, $w = k\dfrac{d^2}{2}$. In this formula w represents the mechanical work, k the constant of the spring and d the distance of extension or compression.

In a series of contractions with a spring instrument such as Cattell's, the loss of power as fatigue comes on is noticeable in the decrease in extent of movement and consequently in a decrease of work and of tension overcome. The decrease, however, is not so rapid or so marked as when the weight ergograph is used.

Isometric Method. — The early investigations of voluntary muscular ability were made with instruments recording only the force of tension overcome by isometric contractions. The method and instruments were almost the same as those employed at the present time in "strength" tests wherein the different forms of the oval dynamometer are used. The manner of experimentation was as follows: an oval dynamometer was grasped in the hand, a maximum squeeze was given, for example, every two seconds, and records were taken in terms of the tension of the spring. The decrease in power as the squeezes continue is very marked and the resultant curve corresponds closely to that obtained with the more recently devised spring ergographs.

II. Critique of the Methods.

In regarding these three types of instruments the investigator notes two sets of objections: those associated with the conception of the instrument and those occurring in the course of experimentation. The first set of objections will be made against the principles of the instruments. The second set of objections will be against the use of the instruments except by the most skilled experimenters. If instruments are used with sufficiently great care many of the latter difficulties will be overcome.

After considering somewhat in detail these three types of instruments it will be admitted, I believe, that all are objectionable in one or both of these particulars. Either the apparatus has been constructed without due regard to the problems for investigation, the principles underlying the type have been wrongly applied, or the instrument is poorly adapted to the work for which it was constructed.

Weight ergograph. With the Mosso ergograph, as has been said,

a weight is lifted, the height to which it is raised is noted and the mechanical work is then calculated. If maximal contractions are repeated every two seconds, at first the extent of movement is great, but after from fifty to one hundred contractions the muscle can no longer raise the weight and no mechanical work is accomplished. The total work, therefore, according to Mosso, which a muscle can do before it is fatigued (exhausted) is the product of the weight and the sum total of the extent of the separate contractions. But is this assumption strictly true? When a muscle can no longer lift a weight, for example, of three kilograms, and consequently under this condition does no mechanical work, can it not do some work? As a matter of fact, Treves[1] and others have found that considerable work may be done if a lighter weight is used. Indeed, the muscle can raise a weight of two kilograms nearly as far as it originally raised the weight of three kilograms. Moreover, when a muscle pulls against a weight it cannot lift, considerable physiological work is done, although the muscle accomplishes no mechanical work. Energy is being used in the generation of heat and of an electro-motive force, and in the production of muscle sounds. This is the main objection that has been urged against the use of a weight — an objection that led Cattell, and Binet and Vaschide to devise their spring ergographs.

Treves[2] has offered a plan for the solution of the difficulty. He advises the use of weights varying with the contractions in such a manner that the maximum amount of work will be accomplished each time. If a method could be devised whereby the fullest extent of contraction be obtained each time and also the greatest possible amount of mechanical work be accomplished, part of the difficulty of the weight instrument would be obviated.

Aside from the mechanical difficulties, which seem for the present insurmountable, there are other difficulties to be considered. For each subject a tedious determination of the conditions recommended by Treves must be made. It would be necessary to determine the greatest weight that could be lifted the greatest distance at the beginning of a series, and to determine the varying weights that would permit a maximum of work throughout a long series of con-tractions. Still another objection may be raised against Treves's recommended procedure, for there is a normal individual variation

[1] TREVES: Archives italiennes de biologie, 1898, xxix. p. 157; 1898, xxx. p. 1.
[2] *Op. cit.*

in the course of fatigue. Consequently, for each new experiment, with its new personal conditions, there must necessarily be used varying masses. This matter has been almost neglected by Mosso and his successors.

In the use of any weight instrument, accordingly, two factors must be considered, the mass and the extent of movement. When an experiment is made with the Mosso ergograph the mass is kept constant, but the extent of the movement decreases as a series continues. Treves, we have seen, would attempt to keep constant the extent of contraction, but would have the mass variable.

The disadvantages of these methods are more evident if we consider the conditions that exist when an attempt is made to compare two or more individuals with one another. Individuals may differ from one another both in absolute force of muscle and in extent of contraction. The muscles of one may be larger — of greater cross section and consequently of greater absolute force — but with a short finger the extent of movement may be comparatively small. In another individual with longer fingers the extent of contraction may be great, but the absolute force of the muscles may be small. If a given weight is used the power measured in kilogram metres may seem greater in the individual with the long fingers but with less muscle. This condition has not been considered by Mosso in the construction of his instrument or in the discussion of results. Treves does not wholly overcome the difficulty although he would allow the muscle to lift the greatest possible weight the greatest possible distance. The varying extent of movement might even in his case permit the muscle of lesser ability to appear as strong as the other.

The changes in extent of movement occurring with the weight ergograph introduce another variable factor that should be eliminated if possible. This factor is the change in nutrition of the contracting muscle. When the movements are long at the beginning of a series of contractions a considerable amount of blood and lymph will be displaced about the muscle tissue. The circulation of the blood and of the lymph will be increased, and the oxidation products of contraction will be taken away readily. When the movements become smaller the circulation changes are not so great, the waste products accumulate and the condition of the muscle is wholly changed. With a constant extent of movement this difficulty will be reduced to a minimum. Such would probably be the case if Treves's plan were followed.

In addition to these grave theoretical faults, the Mosso ergograph is unsuited for the measurement of muscular work except when used by the most skilful experimenters. The inertia phenomena have been noted by several investigators. After the inertia of position has been overcome, the mass acquires an inertia of motion, and may record a movement greater than the extent of the muscular contraction. Often the recorded movement of the weight is not greater than the contraction, but on a careful examination of the separate contraction curves there will invariably be seen at some point of the rise an indentation. This indicates that the mass and the muscle have not travelled together uniformly.[1]

Spring ergograph. The main objection hitherto urged against the use of a weight has been that with a given mass an experiment would show a condition of the muscle in which apparently no work could be done. This objection led Binet and Vaschide, and Cattell, independently, to devise their spring ergographs. They maintain that under normal conditions the muscle never comes to such a state that it can do no work, and that by means of a spring the least muscular force may be registered.

Many of the objections against the use of a weight instrument, however, may be raised with equal force against the use of the spring ergograph. There are, on the other hand, some advantages in favor of the spring ergograph. The inertia phenomena are not so evident. If a strong spring is employed the tension is so great that any momentum is thus overcome. If weak springs are used, the mass is so light that the momentum, depending upon the mass and the velocity, is negligible.

The ergometer of Cattell and, presumably, the spring ergograph of Binet permit at first a maximum contraction of the finger with a maximum of work. As the series of muscular movements continues, the muscular power decreases, there is a decrease in the extent of the movement, and the extension or the compression of the spring lessens. Under these conditions there is almost as much chance for change in nutrition of the muscle as there is with the weight ergograph. The maximal movements at first produce a great increase in the flow of blood and lymph, and the smaller movements after about one hun-

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[1] Curves to illustrate this matter were shown by Professor Warren at the meeting of the American Physiological Society, December, 1898. Similar records were obtained and described at various meetings by Professor Cattell and the present writer, in 1897-8. No detailed account of this work has yet appeared.

dred contractions nearly restore the conditions of normal vascular
supply.

A tacit assumption seems to be made by the users of the various
types of spring ergographs, viz., that the tension of the spring is
immaterial provided the tension constant is sufficiently great to
register a maximum effort each time a contraction is made. Mosso's
experiments, it will be remembered, show that dissimilar masses pro-
duce different characters of fatigue curves and that the mechanical
work accomplished under the different conditions is also variable.
Is a similar effect to be found with the use of springs of different
tensions or may any character of spring be used with similar results?
The following experiments are an attempt to answer this question.

A diagrammatic representation of the apparatus used is shown in
Fig. 1. To a rigid upright (A) were attached springs (B) of dif-
ferent extension constants.
A cord fastened to the other
end of the spring was adjusted
to the muscle employed. In-
directly attached to the cord
connecting the finger to the
spring was a lever (D) that
recorded upon the moving
surface of a kymograph the
extent of the movement of
the spring. The lever was
so arranged as to permit a
magnification of the spring's
movement when such a pro-
cedure was thought advisable. This magnification was often neces-
sary with heavy springs in order that a more accurate reading of the
curves — *i. e.* of the extent of movement of the spring — could be
obtained. The lever was made of aluminum and very light. It was
pointed with a strip of tinsel. Had the lever been left in this simple
condition the inertia of motion might have been sufficient to cause a
distortion of the actual contraction. To overcome this possible source
of inaccuracy the lever was held back by a light spring (E) which was
extended by each movement of the lever. This spring was so weak
in comparison with the extension springs against whose force the
muscle worked, that the work accomplished could be and was en-
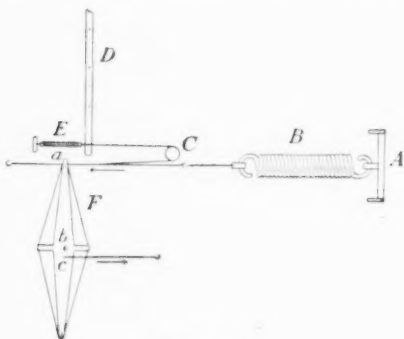tirely disregarded in the calculations. The force exerted to stretch

FIGURE 1.

this small spring was never more than 0.5 per cent of the total force exerted, and often this per cent decreased to 0.1.

The muscle employed was the flexor sublimis of the middle finger of the left hand. The arm was extended in a supine position, the index and the third fingers were placed in closely fitting brass tubes to keep the hand fixed. Straps were placed about the wrist and palm to prevent lateral movements of the arm and hand. The cord attached to the spring was fastened to the finger by means of a metal splint. The two parts of the splint were made to constrict the finger as little as possible; the dorsal part was furnished with an adjustable hook that enabled the experimenter to keep the muscle leverage constant.[1] The constancy of this condition seems not to have been considered in the work of Mosso, Lombard, and Maggiora. In their experiments a leather sling was fitted to the finger and to it the weight cord was attached. In Binet's device the point of leverage is placed opposite the knuckle, but with the artificial axis there must be considerable friction.

FIGURE 2.— Series of 150 contractions with strong spring. The curves read from right to left. The figure is one half the original size of curve.

The three springs employed were a *strong* one with an extension constant of 1 mm. = 1 kg., a *medium* one with extension of 4 mm. = 1 kg., and a comparatively *weak* spring with extension of 15 mm. = 1 kg. The springs were calibrated by the mechanic who made them and by the writer; the variable error of the different springs was not more than the error in reading some of the curves obtained.

Fig. 2 shows a series of contractions with the strong spring. A

[1] This device was suggested to me by the picture of Binet's apparatus (La fatigue intellectuelle, p. 180). After I had begun the construction of my splint, one previously made by Dr. Hough, of the Massachusetts Institute of Technology, was shown me. Dr. Hough's splint is essentially the same as mine. It was exhibited at the meeting of the American Physiological Society, December, 1899. No account of this appears in the Proceedings of that year.

magnification to twice the original extension of the spring was made by the lever in this case for ease in calculation.

In calculating the records of a series of contractions I separated the contractions into groups of tens. The total work accomplished and the total tension overcome in the groups were determined. The work done was calculated by the formula, $w = k\dfrac{d^2}{2}$.

The tension overcome was determined by obtaining the product of the extent of any given movement and the tension constant of the spring used, kd.
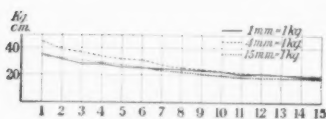
FIGURE 3. — Mechanical work accomplished with different springs. Abscissa denotes number of groups of 10 contractions. Ordinate denotes amount of work.

Fifteen series of one hundred and fifty contractions each were made with each of the springs employed. The averages and the average variation of the groups of ten contractions were calculated and are shown in the accompanying tables and curves.

Considering somewhat in detail the figures in the tables, it will be noted that the different springs permit a maximally working muscle to accomplish various amounts of mechanical work. The spring permitting the greatest amount of work was the one with tension constant of 4 mm. = 1 kg. This result corresponds to what has been found for weights with extirpated muscles. In fifty contractions with a light weight comparatively little work is done, with a medium weight a maximum is accomplished, and with heavier weights there is a decrease in the work done.

FIGURE 4. — Tension overcome with different springs. Abscissa denotes number of groups of 10 contractions. Ordinate denotes tension overcome.

The figures in Table I show, however, that as fatigue comes on the amounts of work with the various springs more closely approximate.

From the variations indicated in the Table we cannot be certain that the differences in work with the various springs are typical.

The results of the tension calculations, on the other hand, are so marked that it cannot be doubted that the outcome is not a chance one. The light spring permitted the greatest movement but re-

TABLE I.

Mechanical work accomplished with different springs. Averages of 15 experiments of 150 maximum movements each. Interval between contractions, 2 seconds.

Groups of 10 contractions.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 mm. = 1 kg.	Work, kg. cm.	342	322	310	290	274	251	239	220	202	194	186	175	175	172	171
	Average variation.	30	60	74	63	56	62	57	54	41	43	39	35	32	34	33
4 mm. = 1 kg.	Work, kg. cm.	456	391	368	335	313	305	276	252	241	221	217	201	192	186	178
	Average variation	85	98	95	90	78	69	66	57	47	45	39	50	45	41	38
1 mm. = 1 kg.	Work, kg. cm.	360	313	283	251	264	255	249	237	230	223	205	204	196	187	184
	Average variation	101	84	76	74	67	72	65	67	73	81	60	68	78	86	83

TABLE II.

Tension overcome with different springs. Averages of 15 experiments of 150 maximum movements each. Interval between contractions, 2 seconds.

Groups of 10 contractions.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 mm. = 1 kg.	Tension, kg.	21.3	20.5	20.2	19.4	18.9	18.1	17.7	17.1	16.4	16.0	15.6	15.3	15.2	15.1	15.2
	Average variation.	0.9	2.0	2.4	2.1	2.0	2.2	2.1	2.0	1.6	1.8	1.6	1.4	1.4	1.4	1.3
4 mm. = 1 kg.	Tension, kg.	47.4	43.7	42.3	40.8	39.1	38.4	36.7	35.0	34.5	32.9	32.7	31.3	30.9	30.1	29.4
	Average variation.	4.1	5.3	5.7	5.6	5.0	4.5	4.5	4.0	3.6	3.5	3.1	4.1	3.7	3.6	3.5
15 mm. = 1 kg.	Tension, kg.	83.2	77.7	74.6	74.1	72.0	70.4	69.6	67.8	66.5	65.3	62.7	62.6	60.3	58.9	58.2
	Average variation.	11.8	10.0	10.0	9.5	9.3	9.8	8.9	9.2	10.5	10.9	10.3	10.4	12.4	13.7	13.7

TABLE III.

Successive series of maximal contractions with different springs. Interval between contractions, 2 seconds. 10 seconds interval between the series.

Groups of 10 contractions	1 mm. = 1 kg.					4 mm. = 1 kg.					15 mm. = 1 kg.					4 mm. = 1 kg.
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1
Work, kg. cm.	26.4	24.2	28.5	23.3	19.0	31.1	23.4	20.8	19.8	18.2	23.3	14.9	17.3	17.0	14.5	21.0
Tension, kg.	72.5	68.0	75.0	67.9	61.1	39.3	34.1	32.2	31.3	30.0	17.5	14.1	15.1	15.0	14.0	32.1

quired the least tension. With the heavy spring there was comparatively little movement, but the amount of tension overcome was reciprocally great.

The objection may be raised that a series of one hundred or more contractions introduces factors that may so affect the character and the force of the contracting muscle that great differences in work and in tension will appear. Such an objection is in itself an admission that the tension constant
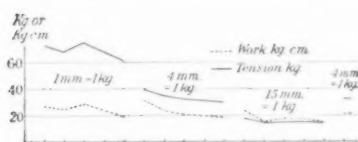
FIGURE 5. — Tension and work in successive series of contractions.

is a factor having a similar effect to the weight in the Mosso ergograph. It is not necessary, however, to use this *argumentum ad hominem.* The objection may be met by having the muscle work successively against springs of different strengths.

If, after a series of contractions against the force of the medium spring, a new series is begun with the light spring, it will be found that the work done with the lighter spring will be greater than that done with the heavy spring. The accompanying tables and curve will illustrate this matter better than a description.

In Table III and in Fig. 5 will be found the results of a series of one hundred and sixty maximum contractions with the three springs used above. The first fifty contractions were made with the strong spring; after ten seconds, during which the spring was changed, a series of fifty contractions was made with the medium spring; and after another ten seconds fifty contractions were made against the weak spring; finally ten contractions were made with the medium spring. In the curve the abscissa denotes the groups of contractions by tens, and the ordinate the units of accomplishment.

TABLE IV.

Successive series of contractions. 10 seconds interval between the series.

	4 mm. = 1 kg.				15 mm. = 1 kg.	
Groups of 10 contractions . .	1	2	14	15	1	2
Work, kg. cm.	69.2	56.8	17.8	12.3	44.1	38.9
Tension, kg.	58.8	53.3	29.7	24.7	25.1	22.6

TABLE V.

Successive series of contractions. 10 seconds interval between the series.

	4 mm. = 1 kg.				15 mm. = 1 kg.		
Groups of 10 contractions .	1	2	3	4	1	2	3
Work, kg. cm.	28.1	26.8	23.3	19.8	31.3	29.6	26.7
Tension, kg.	37.5	36.5	34.0	31.3	20.4	20.0	18.8

Tables IV and V give results of similar experiments upon two different subjects.

These tables clearly show that there is always an increase in the amount of work accomplished whenever a change is made to a lighter spring. This result corresponds to what Treves found when using varying weights. Regarding the figures of tension we note that the results correspond to those in Table II. Almost invariably — there is

TABLE VI.

Alternate maximum contractions with medium and strong springs. Interval between contractions, 10 seconds.

Contractions.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Work, kg. cm. (4 mm. = 1 kg.)	48		45		48		48		43		50		48		41		50
Tension, kg. (4 mm. = 1 kg.)	49		48		49		49		46		50		49		45		50
Work, kg. cm. (1 mm. = 1 kg.)		105		120		120		105		113		128		128		113	
Tension, kg. (1 mm. = 1 kg.)		145		155		155		145		150		160		160		150	

one exception in Table IV — the change from a heavy to a light spring decreases the amount of tension overcome.

One objection may be raised to the consideration of these results as typical. An interval of ten seconds elapsed between the end of a series with one spring and the beginning of the next series with another spring. May not the ten seconds rest suffice for the accomplishment of a greater amount of work after than before the rest? Several series of contractions made with the same spring but with rest intervals of ten seconds indicate that the differences cannot be accounted for in this way. Under the same conditions of spring tension, the amount of work done, or of tension overcome, does not vary more than 15 per cent, while in the tables the variation, with the one exception noted, is from 28 per cent to 258 per cent.

The objection may be answered in a different manner. If movements are made alternately with different springs the conditions will remain relatively constant throughout a long series. The muscle will make a maximum effort each time, and if a difference in mechanical work or in the force of tension overcome is found

TABLE VII.

Alternate maximum contractions with strong and medium springs. Interval between contractions, 10 seconds.

Contractions.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 mm. = 1 kg.	Work, kg. cm.	61		65		53		59		66		65		66		85		65		64		66	
	Tension, kg.	110		114		103		109		115		114		115		130		114		113		115	
¼ mm. = 1 kg.	Work, kg. cm.		109		120		89		97		94		88		85		78		88		85		91
	Tension, kg.		74		74		67		70		69		66		65		63		66		65		68

Contractions.		23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
1 mm. = 1 kg.	Work, kg. cm.	7.4		7.2		64		68		63		63		59		61		62		61		56	
	Tension, kg.	12.2		120		113		117		111		111		109		110		112		110		106	
¼ mm. = 1 kg.	Work, kg. cm.		9.0		8.5		81		80		77		77		76		77		74		73		66
	Tension, kg.		6.7		65		64		63		62		62		62		62		61		61		58

TABLE VII (*continued*).

Contractions	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Work, kg. cm. — 1 mm. = 1 kg.	4.7		5.7		5.5		5.5		6.1		6.2		6.1		6.6		5.8		8.5		5.7	
Tension, kg. — 1 mm. = 1 kg.	9.7		10.7		10.5		10.5		11.0		11.1		11.0		11.5		10.8		13.0		10.7	
Work, kg. cm. — 4 mm. = 1 kg.		7.0		7.7		7.4		7.5		7.8		8.5		7.8		7.7		7.7		7.8		7.5
Tension, kg. — 4 mm. = 1 kg.		5.9		6.2		6.1		6.1		6.3		6.5		6.2		6.2		6.2		6.3		6.1

to exist the cause must be looked for in the use of the different springs.

The following experiment was made to determine whether or not a change corresponding to that found for successive contractions held true for contractions when made alternately. A contraction was first made with the medium spring, ten seconds later the muscle
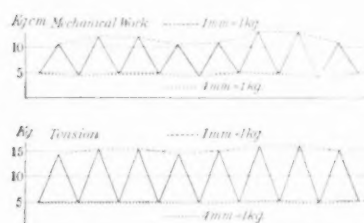

FIGURE 6. — Alternate contractions with different springs. 10 seconds interval between contractions. The successive contractions are joined by unbroken lines. This figure represents the results in Table VI.

contracted against the strong spring and at intervals of ten seconds alternate movements with the springs were made. All the results show similar effects; both the work accomplished and the tension overcome are different when different distension springs are used. The results from only two subjects are here given in Tables VI and VII, and in Fig. 6.

In Fig. 6 the successive contractions are joined by the unbroken line. The work done and the tension overcome with the strong

spring are denoted by the broken line, and the dotted line indicates the work and tension with the medium spring.

In these two experiments, the amount of work accomplished varied for the two springs in different ways for the two subjects. For one subject the medium spring permitted a greater amount of work, for the other subject the heavier spring allowed more work to be accomplished.

The preceding experiments answer the question asked on page 354, viz., whether or not the tension constant of a spring is of minor importance. We have seen that the tension of the spring is of great importance, and that the effect of a change in tension is similar to that observed with changing weights. An isotonic spring seems therefore to be little better than a weight for accurate measurement of the force of a contraction. This conclusion is justified after a consideration of the facts that different springs give discordant results with the same individual, and that different individuals can use different springs to the best advantage, *i. e.* can do more mechanical work or overcome more tension with one spring than with another.

Isometric Spring. — The early investigations upon the force of voluntary muscular contractions, as has been stated, were made with instruments recording only the force of tension overcome by isometric movements. The oval dynamometer to be grasped in the hand is the most familiar of this type of instrument and against this our criticisms will be especially directed.

Two main objections have been raised against the use of this type of instrument: first, the movement is not a simple one, for many muscles are employed; and, secondly, the contractions permitted by the instrument are not maximal in extent. The first objection is a very serious one. In making a complex movement, such as the grip of the hand, many muscles are employed. A slight shifting of the dynamometer will bring into action new unfatigued muscles and will permit others, that have become fatigued, to recuperate partially. This slipping of the dynamometer, due mainly to perspiration, is very apt to occur during a series of movements. In addition to the change in the muscles, a change in the position of the dynamometer may cause a difference in the leverage of the muscles. In consequence there may appear a seemingly greater or lesser power.

The instrument cannot be grasped in precisely the same manner two consecutive times, and results are accordingly not strictly comparable. Moreover, the dynamometer is particularly unsuited for

testing different individuals. Length and contour of hands are so varied that it is almost impossible to keep the conditions constant in the use of this dynamometer.

The second objection noted above — that the contractions are not as great as possible — has for the present writer little force. In fact there are two distinct advantages in the use of such small movements if the other conditions are properly kept constant. If the contractions are practically isometric, the movements are kept uniform in extent throughout a series and the nutrition changes, noted as an objection against the use of isotonic instruments, do not occur. In addition, the extent of movement does not enter as a variable factor as does the force of contraction.

A minor objection to the oval dynamometer is that in making a long series of contractions with it errors are apt to arise from haste in reading the instrument and in recording the force of contractions.

The oval dynamometer on the whole is for use when single determinations only are desired. The difficulty, even the impossibility, of making a series of movements and of attempting to keep the conditions constant would relegate it to that character of research.

A secondary objection, which often becomes primary, against the use of these three kinds of dynamometers is to be made against the loose interpretation of results. All three types of instruments have a value in the determination of certain conditions of muscular activity, but the preceding analyses indicate that these different types cannot be used well for testing muscular force and determining the effects of fatigue.

III. New Application of Isometric Method.

The three methods hitherto used have been found wanting in one or more essentials. The disadvantages of the weight or of an isotonic spring, and of the oval dynamometer have been shown. Where must we look for a solution of the difficulty? The advantage over a weight that a spring possesses must be remembered. Would the application to a spring of the method advised by Treves make conditions more satisfactory, or should we seek another way out of the difficulty? The objections to Treves' method for a weight are equally potent against its application to a spring, although, if the mechanical difficulties were overcome, the method might be a valuable one.

We are compelled, therefore, to seek further. If the conditions of experimentation are considered, we see that there should be the least possible change in the vascular condition of the muscle, and at the same time the method should permit a comparison of results for different individuals, and for the same individual at different times.

The inertia problem, we have seen, is least evident when a spring is used. The changes in the extent of movement and the consequent change in nutrition of the muscle may be obviated only by keeping the extent of movement constant. Treves' suggestion, as we have seen, is impracticable. The use of isometric movements — movements of equal but minimum extent — immediately suggests itself as an alternative. The oval dynamometer, it will be recalled, had among its great disadvantages this one advantage of keeping practically constant the extent of contraction of the muscle. Can this method be used, therefore, and the faults of the oval dynamometer be eliminated?

Two ways of easily accomplishing this result immediately suggest themselves. The simpler method is to attach a strong flat spring to a rigid base and have the muscle work against the portion of the spring near the base.[1] If the muscle leverage and other factors are kept constant this device is thoroughly satisfactory. The other method is to attach to an ordinary expansion spring a reducing lever in such a way that the muscular movement may be practically isometric although the spring's movement will be comparatively large. A diagrammatic representation of this apparatus is found in Fig. 1, p. 354. To the
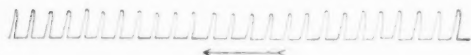

FIGURE 7. — Contractions with spring. Interval, 2 seconds. Magnification of muscular movement, 50. Curve, actual size.

1 mm. = 1 kg. spring (B) was attached the reducing lever (F) which worked upon a knife-edge fulcrum, *b*. The muscular power was applied at the point *c* by means of a cord attached to the finger. The spring was extended by the movement of the lever at *a*. The movement of the spring was ten times as great as the movement of the muscle, and for ease in reading, the curves of the extension of the spring were magnified five times by means of the writing lever (D).

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[1] An illustration of this apparatus designed for use with the abductor indicis will be found in the Introduction to Physiology, by Dr. W. T. Porter, Boston, 1900.

TABLE VIII.

Isometric maximal contractions. Interval between contractions, 2 seconds.

Groups of 10 contractions.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tension, kg.	.94	.91	.81	.80	.77	.67	.59	.60	.57	.53	.52	.46	.47	.42	.42
Average variation	.05	.1	.2	.1	.1	.05	.2	.4	.3	.2	.1	.1	.04	.1	.1

All the other conditions of the experiment were similar to those described in a previous section of this paper.

Fig. 7 gives a portion of a series of contractions made in this manner. The actual movement of the finger was only one-fiftieth of the extent of the movement recorded. For all practical purposes, therefore, the movements at the beginning and at the end of a series of one hundred and fifty contractions are equal in extent although unequal in strength.

In Table VIII will be found the average amount of tension overcome and the average variation with this isometric method.

A glance at the conditions of the experiment and at the results will indicate some of the advantages of the isometric method employed.

1. The spring type of instrument has been adopted because, unlike a weight, the spring permits a record of force expended according to the state of the muscle. With the weight ergograph the weight is often not lifted when the muscle can still accomplish a large amount of mechanical work. The muscle or nerve cells will almost never be thoroughly fatigued and incapable of work,[1] and with the spring the least force of the muscle may be determined.

2. The inertia of motion, so troublesome a factor when a weight is used, is obviated by the use of a strong spring.

3. The nutrition changes consequent to the change in extent of movement are less apt to be a disturbing factor with the

[1] Occupation neuroses are exceptions. In these cases the muscles seem to be normal but the nervous mechanism is exhausted.

condition advised. With the minimum movements the circulation of the blood and of the lymph will not be greatly altered.

4. The isometric method not only keeps constant the conditions throughout the experiment, but the extent of movement is constant throughout a number of experiments.

5. Moreover, the movement is of equal extent for different individuals. The length of the muscle or of the fingers does not enter as a disturbing factor. Practically the absolute force of the muscle is measured, and this factor for experiments on fatigue is of more importance than the extent of movement.

6. Convenience and ease of construction are minor factors, but these, too, are not lacking.

IV. FATIGUE.

In Tables I, II, and VIII there is material for an estimation of the effect of repeated contractions upon muscular work. Mosso, Maggiora, Lombard, and others found that after from 50 to 100 contractions a muscle could not lift the different weights used. The extent of the movement decreased from a maximum to a minimum, and a corresponding change took place in the amount of work. In the present experiments with springs, after the muscle had been contracted 150 times it was still able to do considerable mechanical work. The relative amount varied with the different springs employed, but seldom was the muscle incapable of performing at the close of 150 contractions at least 40 per cent of the amount it was able to accomplish at the beginning of a series. For convenience the average work and the average tension overcome in the first, eighth and fifteenth groups of contractions are shown in Table IX. Fig. 3 and Fig. 4 show the course of fatigue with the isometric springs, 15 mm. = 1 kg., 4 mm. = 1 kg., and 1 mm. = 1 kg.

The results in this table indicate that the greatest loss of power takes place during the first eighty movements. After that point the loss is more gradual and more regular. Nearly always the loss in power is more marked during the first fifty movements than in any subsequent portion of the series. These results, it will be noticed, are not in accord with the results obtained with weights. In the weight curve, although there are slight individual differences, the decrease in power at first is least and during the final twenty or thirty movements the loss is very great.

Similar effects are noticed if instead of making successive move-

ments, a voluntary tetanus is maintained against the force of a spring or against a weight. With the weight the fall at first is very gradual and towards the end the power seems to be lost suddenly. With a spring the curve corresponds closely to the curve for successive contractions. At first there is a rather sharp decline, followed by a more gradual decrease in the height of the tetanus curve. The accompanying reproduction of such a curve, in Fig. 8, with the medium spring will show the gradual fall of the tetanus.

TABLE IX.

Effect of fatigue upon muscular power. Maximal contractions. Interval between contractions, 2 seconds. Figures are averages of 15 experiments under each condition.

Groups of 10 contractions.		1	8	15
15 mm. = 1 kg.	Work, kg. cm.	34.2	22.0	17.1
	Tension, kg.	21.3	17.0	15.2
4 mm. = 1 kg.	Work, kg. cm.	45.6	25.2	17.8
	Tension, kg.	47.4	35.0	29.4
15 mm. = 1 kg.	Work, kg. cm.	36.0	23.7	18.4
	Tension, kg.	83.2	67.8	58.2
Isometric.	Tension, kg.	9.4	6.0	4.2

The following figures from two tetanus curves will illustrate the results obtained : —

	I.	II.
Extent of contraction at first	23.3 mm.	21.3 mm.
Extent of contraction after 100 seconds	14.2 mm.	14.6 mm.
Extent of contraction after 200 seconds	10.3 mm.	10.3 mm.
Extent of contraction after 300 seconds	7.0 mm.	7.0 mm.

After glancing at the figures, an interesting question immediately suggests itself: What relation does the work or the tension bear to this decrease in power? The answer to this question is rather a
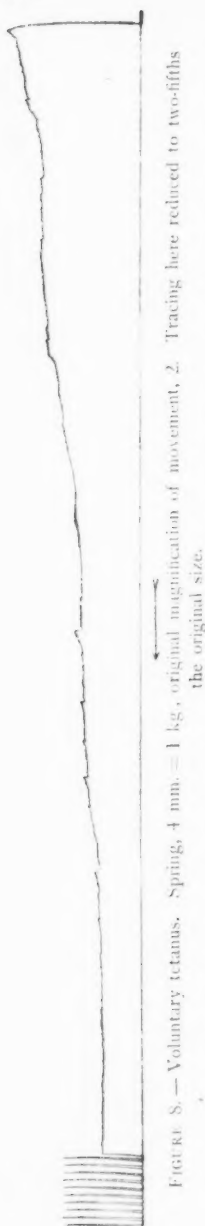
Shepherd Ivory Franz.

matter for investigation than for discussion at the present time, but a consideration of the conditions present may serve to indicate whence the answer must be sought.

What probably happens in such an experiment is that the muscle is constantly relaxing and contracting to a trifling extent about fifteen times a second. The amplitude of vibration may be judged to be not more than 0.1 mm. since it cannot be seen in the magnified curves. If these conditions are assumed to exist it will be found that the amount of mechanical work accomplished equals, if it does not surpass, in amount that which is done when single contractions are made. The tension overcome in the tetanus curve for two seconds is probably greater than that overcome in the single movement made every two seconds.[1]

Throughout the previous portions of this paper we have considered only what are average or typical results. It must not be for-

[1] The accurate calculation of the tetanus curve is difficult and tedious. For the present purpose the curve may be considered a simple one wherein there is a rapid rise and a gradual and regular fall. The calculation of work and tension may be further simplified by considering the curves at different points, for example every ten seconds, and making calculations at these points.

Let the accompanying figure represent the condition of an experiment. a is the spring not distended, b the amount of distension in tetanus, and c the distance of vibration due to tetanus. Since the variation in force is not proportionately great we may consider the force as constant; and the work accomplished in one vibration from x to y would be kcb.

The tension overcome in one vibration is the tension to b + tension to c: $tension = k (b + c)$. But since c is very small compared to b it may be disregarded, and we get $tension = kb$.

gotten, however, that the processes considered are quite variable. Mosso and his students seem to have drawn conclusions from one experiment and have disregarded the possibility of a *normal* variation. Tables I, II, and VIII show the amounts of variations in work accomplished and in tension when different springs were used. Fifteen experiments of each kind were made. The conditions of the experiment were kept as constant as possible; the observer felt well during the progress of the series; the experiments were made at the same time each day; the temperature of the body and of the surrounding atmosphere was practically constant; but the barometric conditions and humidity varied. No series was made upon any day when the subject felt unable to give full attention to the experiment or when he felt unable to do a maximum amount of work. Notwithstanding the effort to have the physiological and mental conditions as constant as possible a considerable variation was found to exist. In many cases this amounted to one-quarter of the total measurement. The reason for this great variation is not evident and the topic needs investigation.

The individual variations are well marked, but in the present series not sufficient data have been obtained to warrant the drawing of any conclusions. All that may be safely said at present is that the curves obtained show an individual peculiarity both in the form of the curve and in a difference in amount of work and tension.

V. Conclusions.

The foregoing article is largely a destructive critique of the methods hitherto used for measuring muscular ability. The constructive portions are devoted to the description of a new method which seems to have few or none of the disadvantages of the older methods and to some general conclusions regarding the course of fatigue.

The following conclusions seem to be justified from the results of the experiments and analyses : —

1. The isotonic use of a weight or of a spring for measuring muscular force is not justified because two variable factors — extent and force — are introduced.

2. The assumption that the weight × height is a measure of muscular power cannot be defended.

3. Results obtained with springs of different extension constants show either that the work that can be accomplished or the tension

that can be overcome varies with the extent of the movement, or that the isotonic spring is not a good measure of muscular ability.

4. For comparative results upon different individuals and upon the same individual at different times a weight or a spring should not be employed in an isotonic manner.

5. The isometric use of a spring possesses the advantages lacking in the use of other kinds of instruments.

6. The fatigue curves obtained by Mosso and later investigators with weights do not represent the true state of the neuro-muscular mechanism.

7. The amount of work that can be accomplished, measured by springs of different extension constants, is about 40 per cent as great at the end of a series of 150 contractions as at the beginning.

8. Under similar objective conditions the daily variation is large.

THE REACTIONS OF PLANARIANS, WITH AND
WITHOUT EYES, TO LIGHT.

BY G. H. PARKER AND F. L. BURNETT.

CONTENTS.

I. INTRODUCTION.

THAT the movements of planarians are largely influenced by light
seems to have been first pointed out by Loeb [1] (1893, p. 101;
1894, p. 255),[2] who showed that in the case of Planaria torva the
animals were stimulated to locomotor movements by light and came
to rest only in places where the light intensity was greatly reduced.
He further showed that the same reactions could be observed in
animals deprived of their eyes, though in such individuals the re-
action time was much longer than in those with eyes. Hesse (1897,
p. 550),[3] apparently without knowledge of what Loeb had done,
made similar experiments on Planaria gonocephala and obtained
results that in the main confirm Loeb's.

It is the purpose of the present paper to show more fully than has
been done heretofore, the relations of planarians with eyes to those
without eyes in their reactions to light. The question whether these

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[1] The complete references to articles cited will be found at the end of this
paper.

[2] LOEB: Archiv für die gesammte Physiologie, 1893, liv, p. 101; 1894, lvi, p.
255.

[3] HESSE: Zeitschrift für wissenschaftliche Zoologie, 1897, lxii, p. 550.

reactions are to be interpreted as photopathic or phototactic is intentionally reserved for later consideration.

The species chosen for study was Planaria gonocephala Dugès, and the following method of experimenting was adopted. A planarian was placed in a shallow, rectangular glass dish containing water to the depth of about one centimetre. After the animal had taken hold of the glass surface and had begun to creep, the dish was placed on a black board on which was inscribed a circle whose diameter was 55 millimetres. This was divided into quadrants by mutually perpendicular diameters, and the arc of each quadrant was further divided into intervals of ten degrees, by short cross lines in the circumference. These lines were designated in degrees, the one at the end of one of the diameters being taken as zero and those in the semicircles to the right and to the left of this zero being numbered in corresponding series till they met at 180. By moving the dish containing the planarian, the animal, without serious disturbance to its movements, could be placed with its centre over the centre of the circle and with its head directed toward the point marked zero. The anterior end of the animal was consequently somewhat beyond the centre of the circle, and we endeavored to have the animal so placed that a straight line from the anterior tip of the head to the circumference measured 25 millimetres. This line, which was a part of a radius of the circle, marked the shortest course the animal could take in reaching the circumference. The apparatus thus arranged was set up in a chamber protected from extraneous light, and illuminated by a Welsbach burner, 25 centimetres from the middle of the dish. Between this source of light and the dish was placed a glass vessel having flat sides 4 centimetres apart and containing a saturated solution of alum to absorb the heat rays. Light was made to enter the dish either horizontally through its flat vertical side only, or, by means of an appropriate screen and reflector, vertically from above. Thus the animals, whose movements were limited to a horizontal plane, could be subjected to the action of horizontal or of vertical light.

In all, six sets of experiments were performed, and in each set ten animals were tested, each one five times. In each trial the animal was set as already described at the middle of the circle and its movements, until it crossed the circumference, were observed. The time it required in moving from the centre to the circumference was taken in seconds by a stop-watch; its course was marked free hand on a

duplicate circle during the locomotion and afterwards measured in millimetres, thus giving the approximate distance it travelled in passing from the centre to the circumference; and, finally, the angle at which the circumference was crossed was recorded in degrees. Thus for each trial three records were made: time, distance, and angle.

The six sets of experiments were carried out under the following conditions. The first three were on animals with eyes, and in the first of these the dish was illuminated with horizontal light and the animal was directed toward the source of light. In the second, the dish was again illuminated with horizontal light, but the animal was directed away from the source of light. In the third, the dish was illuminated with vertical light and the animal directed toward the zero of the circle. The three remaining sets were repetitions of those just described, except that they were carried out on eyeless animals. The removal of the eyes was accomplished by cutting off the anterior ends of the animals with a sharp scalpel, an operation that, as is well known, is attended apparently with only very slight disturbances in the animals. In all cases, however, at least 24 hours were allowed to elapse after the operation before the animals were subjected to experimental tests.

II. Experiments.

In the first set of experiments, as mentioned above, planarians with eyes were subjected to the action of horizontal light, being directed towards its source. That some idea of the general character of the records obtained in these experiments may be gained, the readings from this first set of experiments are reproduced in full in Table I. Here the ten animals experimented upon are designated each by a letter, and the fifty trials to which they were subjected are numbered consecutively from 1 to 50. The columns marked "Left courses" give the records of those instances in which the animals turned to the left from a straight path towards the light, *i.e.* exposed the right sides of their bodies to full light; and the columns marked "Right courses" give the records of those cases in which the animals turned to the right. Under each of these headings are placed the results of the observations as to angle, distance, and time. The table shows that in the 50 trials, the animals turned 27 times to the left and 23 times to the right. The average angle of emergence at the circumference was on the left 87.3°, on the right 66.7°: the average distance traversed was in the left courses 35.6 millimetres,

TABLE I.

Individual Animals.	Number of the trial.	Left courses.			Right courses.		
		Angle of emergence in degrees.	Distance in mm.	Time in seconds.	Angle of emergence in degrees.	Distance in mm.	Time in seconds.
A	1	100	34	44			
	2	70	30	30			
	3	45	34	42			
	4	. . . .	. . . .	. . . .	90	28	35
	5	10	28	29			
B	6	2	32	30			
	7	. . . .	. . . .	. . . .	90	40	42
	8	120	33	29			
	9	55	30	28			
	10	. . . .	. . . .	. . . .	75	35	30
C	11	125	35	30			
	12	117	36	27			
	13	. . . .	. . . .	. . . .	50	42	31
	14	. . . .	. . . .	. . . .	70	30	25
	15	65	31	25			
D	16	87	30	51			
	17	. . . .	. . . .	. . . .	25	34	40
	18	108	36	45			
	19	140	33	39			
	20	80	35	49			
E	21	110	34	27			
	22	. . . .	. . . .	. . . .	55	37	30
	23	. . . .	. . . .	. . . .	20	31	26
	24	102	34	29			
	25	. . . .	. . . .	. . . .	45	33	28

TABLE I (*continued*).

Individual Animals.	Number of the trial.	Left courses.			Right courses		
		Angle of emergence in degrees.	Distance in mm.	Time in seconds	Angle of emergence in degrees.	Distance in mm.	Time in seconds
F	26	90	38	29			
	27				85	31	35
	28	120	42	34			
	29	120	42	34			
	30	100	43	45			
G	31				70	30	32
	32	70	30	24			
	33				80	36	30
	34				35	31	27
	35				50	38	25
H	36				25	86	90
	37				107	53	49
	38	115	63	54			
	39				100	49	40
	40				130	45	42
I	41				90	31	31
	42				30	30	39
	43	60	34	37			
	44				40	32	33
	45	75	35	34			
J	46	75	31	28			
	47				80	35	33
	48	100	40	32			
	49	95	38	30			
	50				95	36	33
Averages		87.3	35.6	34.6	66.7	38.0	35.9

in the right 38.0; and the average time consumed in reaching the circumference was in the left 34.6 seconds, in the right 35.9. Combining the observations of the right and of the left courses, it will be found that the general average position of emergence was at 77.86°, the average distance traversed to reach this position was 36.68 millimetres, and the average time consumed in passing over this distance was 35.22 seconds. From the data for these last two statements, it can be calculated that the animals moved at the average rate of 1.04 millimetres per second.

TABLE II.

Condition of animal.	Direction of light.	Original direction of animal.	General averages.			
			Angle of emergence in degrees.	Distance in mm.	Time in seconds.	Rate in mm. per second.
With eyes.	Hori-zontal.	Toward light.	78	37	35	1.04
		Away from light.	24	28	25	1.12
	Vertical.	Toward zero of scale.	27	27	25	1.08
Without eyes.	Hori-zontal.	Toward light.	57	36	44	0.82
		Away from light.	35	32	36	0.89
	Vertical.	Toward zero of scale.	39	33	38	0.87

The observations for the remaining five sets of experiments were collected and dealt with as in the first set. Since the general averages are all that are needed, it is not necessary to state the records of these experiments in detail, but their results may be presented in a condensed form. This has been done in Table II., which also includes similar statements from the first set of experiments.

Except in the column for rates, the averages are expressed in whole numbers. The records of the angles of emergence represent deviations from zero, which was the point on the circumference toward which the animals were in all cases directed.

III. DIRECTIVE INFLUENCE OF LIGHT.

Planarians with Eyes. — The action of light in influencing the direction of movements of planarians can best be made clear by some such graphic method as that used in Fig. 1. The right half of this figure represents results obtained from the first set of experiments (planarians in horizontal light and directed toward its source). The semicircumference of this half is imagined divided into arcs of 10° each, and each arc is marked by a number corresponding to the designation of its middle point in degrees; thus the arc 0 to 10 is marked by its middle point 5, 10 to 20 by 15, etc. From each middle point a portion of a radius is drawn, the length of which is proportional to the number of times that arc was passed over by outgoing planarians; thus the arc between 0 and 10 was passed over twice, and that between 10 and 20 once, hence the line drawn from 5 is twice as long as that drawn from 15. The numbers of cases

FIGURE 1. — Distribution of points at which planarians with eyes passed over the circumference of the circle, (1) when affected by horizontal light and directed toward its source (right half of figure), and (2) when under vertical light and directed toward 0° (left half). The method of constructing the figure is described in the text.

upon which the lengths of the lines depend are given at the inner ends of the lines. By connecting these inner ends, a curve is produced that gives some idea of the frequency with which different parts of the circumference were passed over. It must be borne in mind that, in constructing this figure, the records of both right and left courses have been compiled together on the right semicircle and that, therefore, the figure represents a general statement and not the condition for the right side only. An inspection of the figure shows that planarians starting from the centre of the circle toward the source of light passed over the circumference anywhere
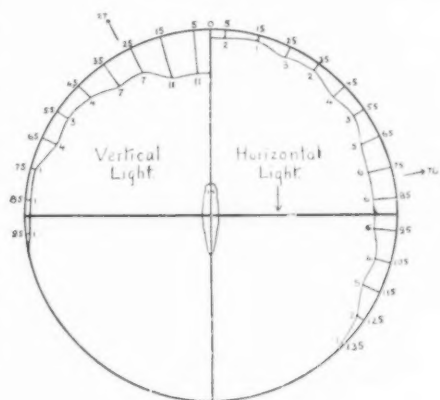
between 0° and 140° and that the region of most frequent emergence was between 70° and 100°, the position of average emergence being 78°.

The extent to which this condition is due to the directive influence of light can be judged from the third set of experiments, the results of which are exhibited in the left half of Fig. 1. These show the effects of vertical light. Here, of course, the directive influence of the light is rendered ineffective, and the animals, pointed toward zero, often cross the circumference very close to that point. Failures to do so are probably to be attributed to some slight internal or external deflecting influence other than light. The extent to which these accidents may affect the animals' movements is shown in the figure. The circumference was crossed anywhere between 0° and 100°, the most usual region being 20° either side of zero. Combining the observations from the right and the left sides as in the first set of experiments, the position of average emergence is found to be 27°.

Comparing the results of the first set of experiments, in which the light exerted its full deflective action, with those of the third set just described, in which that action was eliminated, it is evident that the portion of the circumference over which the animals passed was increased by the deflective action of the light from 100° to 140°; that the region of most frequent emergence was moved from the interval between 0° and 20° to that between 70° and 100°; and finally that the position of average emergence was changed from 27° to 78°. The direction of all these changes, as might be expected, was away from the source of light.

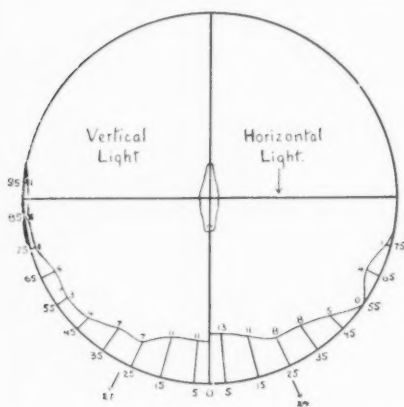In the second set of experiments, planarians were subjected to

FIGURE 2 — Distribution of points at which planarians with eyes passed over the circumference of the circle, (1) when affected by horizontal light and directed away from its source (right half of figure), and (2) when under vertical light and directed toward 0° (left half). For further explanation see page 379.

horizontal light, but were directed away from its source. Their resultant movements are tabulated on the right side of Fig. 2, the left side of which is an inverted duplicate of the corresponding side of Fig. 1 and is reproduced for convenience of comparison. The figure shows that the animals moving in the same direction as the light, passed over the circumference between 0° and 80°, that the region of most frequent emergence was between 0° and 10°, and that the position of average emergence was at 24°.

In comparing these results with those obtained in vertical light, it is obvious that the horizontal light has acted in a restrictive way. The portion of the circumference over which the animals passed has been reduced from 100° to 80°; the region of most frequent emergence was 10° either side of zero instead of 20°; and the position of average emergence was removed from 27° to 24°. The movements of the animals were thus limited to a narrower field.

The conclusions to be drawn from the three sets of experiments on planarians *with* eyes are, first, that planarians moving horizontally *toward* a source of light are deflected further from an ideal course (to 0°) than when moving under

FIGURE 3. — Distribution of points at which planarians without eyes passed over the circumference of the circle, (1) when affected by horizontal light and directed toward its source (right half of figure), and (2) when under vertical light and directed toward 0° (left half). For further explanation see page 379.

vertical light, and, secondly, that when moving horizontally *away* from a source of light, they are kept more closely to an ideal course than when moving under vertical light. It is also to be noticed that the effect of horizontal light is much greater on animals started toward than on those started away from the source of light. These are the natural consequences of a peculiarity which these animals possess of moving away from a source of light when the rays fall upon them in an effective direction.
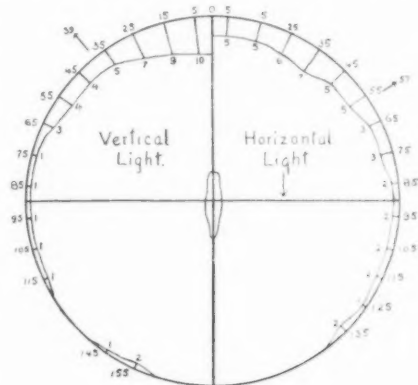
Planarians without Eyes. — To what extent planarians without eye
react toward light can be seen from the remaining three sets of
experiments. The first of these, like the first one on planarians with
eyes, was carried out in horizontal light with the animals directed
toward the source of light. The records are given on the right side
of Fig. 3. The circumference was passed over anywhere between
0° and 140°; the region of most frequent emergence was not very
clearly marked, but presumably lay between 30° and 40°; and the
angle of average emergence was 57°.

The reactions of eyeless animals in vertical light are tabulated in
the left half of Fig. 3. The portion of the circumference passed
over extended, in scattering cases at least, as far as 160°. The
region of most frequent emergence was between 0° and 10°, and
the angle of average emergence was 39°.

Comparing the results of the previous set of experiments with
these to ascertain what influence horizontal light may have had, it
is clear that, so far as the extent of circumference passed over is
concerned, no noteworthy difference is to be observed. The region
of most frequent emergence, however, was moved from the interval
between 0° and 10° to that between 30° and 40°, and the angle of
average emergence was shifted from 39° to 57°. These two changes
are similar in direction to the corresponding changes observed in
planarians with eyes.

Horizontal light also affects the courses of eyeless planarians
moving away from its source. This is shown in the right half of
Fig. 4. As the scattering cases indicate, apparently any part of the
circumference may be crossed by outgoing planarians, though the
majority of positions lie between 0° and 80°. The region of most
frequent emergence was between 0° and 10°, and the angle of aver-
age emergence was 35°.

The effect of horizontal light, in determining the extent of circum-
ference made use of in emerging, was in this case, as in the former,
not very pronounced. The region of most frequent emergence was
the same as in vertical light (0° to 10°), but it was represented in
horizontal light by 16 cases as against 10 in vertical. The angle
of average emergence was moved from 39° to 35°. Where changes
were noticeable they were in the same direction as already observed
in planarians with eyes.

These experiments demonstrate that planarians without eyes are
considerably influenced in their movements by light, for they keep

more exactly to a course when moving with the light and turn more extensively from a course when moving against it, than can be accounted for by accidental meeting with other stimuli.

We have seen nothing in our experiments that supports the opinion suggested by Hesse (1897, p. 551),[1] that reactions such as we have described are due to the direct influence of light on the internal parts of the planarians, and we are more inclined to the view that these reactions are initiated by the effect of light on the integument of the animal, *i.e.* are due to what Graber (1883, p. 229 ,[2] has called a dermatoptic function.

Comparison of Reactions of Planarians with and without Eyes. — The preceding experiments show that planarians without eyes reacted to the directive influence of light in much the same way as those with eyes, but with less precision and often to less extent. Thus, animals with eyes when directed toward the light, had their angle of average emergence shifted from 27° to 78°; those without eyes, from 39° to 57°, a change of very much less extent. The reverse seemed to be true

FIGURE 4. — Distribution of points at which planarians without eyes passed over the circumference of the circle, (1) when affected by horizontal light and directed away from its source (right half of figure), and (2) when under vertical light and directed towards 0° (left half). For further explanation see page 379.

when the animals were directed away from the light, for those with eyes then changed their angle of average divergence from 27° to 24°, those without, from 39° to 35°, a greater difference. The fact, however, that in this case both changes were slight and that the regions of change were in different parts of the circumference, may make this exception more apparent than real, though this question cannot be settled from our present observations.

[1] HESSE : Zeitschrift für wissenschaftliche Zoologie, 1897, lxii, p. 551.

[2] GRABER : Sitzungsberichte der kaiserliche Akademie der Wissenschaften Wien, 1883, lxxxvii, p. 229.
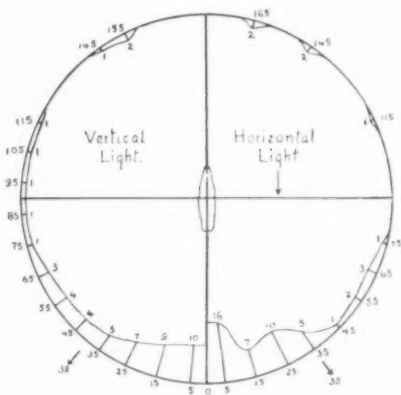
The decrease in precision of the movements of eyeless animals as compared with that of animals having eyes, appears in two ways. First, the animals with eyes cross the circumference of the circle in well-circumscribed regions, while those without eyes, besides showing regions of maximum crossing, also often cross in very remote positions, thus giving evidence of a wandering tendency (compare Figs. 1 and 2 with 3 and 4). The decrease of precision in eyeless animals is also indicated in the fact that in vertical light the angle of average emergence for these animals is 39° and for those with eyes 27°. The reason planarians under vertical light and moving towards 0° do not reach that point is because of the influence of numerous small deflecting stimuli other than light. These stimuli when excessive give rise to wandering courses, and, as the angle of average emergence gives a rough measure of these irregularities and is greater in planarians without eyes (39°) than in those with eyes (27°), it follows that those without eyes must have moved with less precision.

IV. RATE OF MOVEMENT.

As an inspection of Table II will show, planarians without eyes under all the conditions of our experiments move more slowly (0.82 mm. to 0.89 mm. per sec.) than those with eyes (1.04 mm. to 1.12 mm. per sec.). It is not possible to state with absolute certainty that this condition is due simply to the loss of eyes and that it is not in some other way a direct effect of the operation; nevertheless the facts that it was persistently characteristic of the eyeless specimens and that the operation had in other respects so slight an influence lead us to believe that it is in the main due to the absence of eyes.

In both kinds of planarians, the rate of travel is slowest in animals under the influence of horizontal light and directed towards its source (1.04 mm. per sec. and 0.82 mm. per sec.). This is probably due to the fact that these animals moved in a curve, and not in a nearly straight line, as in the other experiments. The animals that showed the most rapid rates of movement (1.12 mm. per sec. and 0.89 mm. per sec.) were, as might have been expected, those that moved with the light. Thus the rate of movement is dependent primarily upon the presence or absence of eyes, and secondarily upon the direction in which the animal is moving with reference to the source of light.

V. CONCLUSIONS.

Planarians without eyes react to the directive influence of light in much the same way as those with eyes, in that they have a tendency to turn away from the course when directed toward the source of light and to keep in it when directed away from the source, though with less precision and often to less extent than planarians with eyes.

Planarians with eyes move more rapidly (1.12 mm. to 1.04 mm. per sec.) than those without eyes (0.89 mm. to 0.82 mm. per sec.); and those moving away from the light (1.12 mm. and 0.89 mm. per sec.) than those moving toward it (1.04 mm. and 0.82 mm per sec.).

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FURTHER EVIDENCE OF THE POISONOUS EFFECTS OF A PURE NaCl SOLUTION.

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IN testing his conclusion that a pure solution of an electrolyte is a poison, Loeb found that Fundulus, a marine fish, could not live in a pure NaCl solution of the same concentration as sea-water.[1] As it is quite probable that the proteid composition of the tissue of marine and fresh-water animals differs, Professor Loeb suggested that I repeat these experiments upon fresh-water forms in order to determine whether the poisonous quality of a pure NaCl solution is of a general character. Two questions were to be answered in this connection. Is Na a poison? Does Ca counteract its ill effects? To both of these I obtained an affirmative answer — results in perfect accord with those of Loeb.

MATERIAL AND METHODS.

The experiments were performed upon Trout hatched in the laboratory. The young fish are especially good for these experiments. They are hardy, and live without difficulty if kept in running water. The yolk sac is not absorbed for from forty to sixty days, so that it is not necessary to feed them; in addition they are comparatively large and vigorous when hatched. It is therefore an easy matter to determine when changes produced by the solutions take place.

The solutions were put into large, covered, Stender dishes. These were kept standing in running water of about 10° C. to obtain a favorable temperature, and were opened from time to time to renew the oxygen supply. Practically the only difficulty met with in handling the material arose from the fact that the salt solutions cause an increased secretion of mucin from the body surface. This often hardened into threads which caught dust particles. The current

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[1] LOEB, J.: This journal, 1900, iii, p. 331.

made by the gill motion attracted the mass into the mouths of the specimens, and they were choked. By careful watching, however, this difficulty could be avoided and the threads could be removed with fine forceps before harm was done. The series of experiments was begun a few days after the young fish were hatched, and extended over a period of two months. The increasing age of the fish seems to present little difficulty in the comparison of results, for the experiments have not shown that constant variation in a definite direction which might have been expected.

The series of changes which result in death are practically the same in the different solutions. First there is a change in the character of the swimming motions; the fish shows a tendency toward circus motions when stimulated, and these are combined with a bending of the body from side to side and a lashing movement of the tail. Soon after this all motion of the body ceases, and breathing becomes difficult. Breathing then ceases, and after an interval the heart stops beating. It is worthy of note that *cessation of respiration invariably precedes cessation of the heart-beat.* The length of time varies. In many cases it may not be longer than a few minutes, but it is often as long as two or three hours. In some solutions, after the heart has stopped beating the tail continues to live, and for many hours contracts rhythmically. In order to secure a uniform basis for comparison, cessation of respiration was considered the mark of death, and length of life was calculated on that basis.

THE EFFECT OF NaCl IN PURE SOLUTION AND IN COMBINATION WITH CaCl₂ AND KCl.

If the young fish are placed in a series of solutions in which NaCl has been added to water in gradually increasing quantities, it will be found that (1) in distilled water they live indefinitely (one specimen lived 51 days); (2) in very weak solutions they continue to live indefinitely, for the osmotic pressure is too small to cause the salt to enter the tissues in sufficient quantity to do harm; (3) at a certain concentration the poisonous effects of the salt assert themselves, and the fish die in a comparatively short time; (4) in very strong solutions osmotic pressure is so great that death is caused almost at once by osmotic effects which are quite independent of ionic effects. As there are no poisonous effects in very weak solutions, no further experiments were tried with them. In strong solutions it is possible

to prolong life several hours by the addition of Ca, but of cours[e]
ionic effects are complicated by the effects of osmosis. The strength[s]
in which the poisonous effects just assert themselves ($\frac{2}{3}$ n and $\frac{1}{2}$ n) ar[e]
then most favorable for testing the neutralizing power of Ca. I[f]
CaCl$_2$ is added to NaCl solutions of these strengths it is found tha[t]
life continues much longer than in a pure solution. It is therefor[e]
safe to conclude that Ca has the power to neutralize the poisonou[s]
effects of Na. The following table gives evidence of these facts : —

TABLE I.

Brown Trout.

Pure NaCl solutions.	Duration of life.		NaCl solutions + CaCl$_2$.	Duration of life	
	Max.	Av.		Max.	Av
100 c.c. $\frac{2}{3}$ n NaCl	2 hrs.	1$\frac{1}{2}$ hrs.	100 c.c. $\frac{2}{3}$ n NaCl + 2 to 5 c.c. $\frac{10}{2}$ n CaCl$_2$	3$\frac{1}{2}$ hrs.	3 hrs.
100 c.c. $\frac{1}{2}$ n NaCl	2$\frac{1}{2}$ hrs.	2 hrs.	100 c.c. $\frac{1}{2}$ n NaCl + 2 c.c. n CaCl$_2$	6 hrs.	
100 c.c. $\frac{1}{4}$ n NaCl	4 hrs.	3$\frac{1}{2}$ hrs.	100 c.c. $\frac{1}{4}$ n NaCl + 2 to 10 c.c. $\frac{1}{2}$ n CaCl$_2$	13 hrs.	
100 c.c. $\frac{1}{10}$ n NaCl	22 hrs.	14 hrs.	100 c.c. $\frac{1}{10}$ n NaCl + 10 to 12 c.c. $\frac{1}{2}$ n CaCl$_2$	40 hrs.	33 hrs.
100 c.c. $\frac{2}{3}$ n NaCl	17 da.[1]	45 hrs.	100 c.c. $\frac{2}{3}$ n NaCl + 12 c.c. $\frac{1}{4}$ n CaCl$_2$	37 da.	3 da.
100 c.c. $\frac{1}{2}$ n NaCl	23 da.	12 da.	100 c.c. $\frac{1}{2}$ n NaCl + 8 c.c. $\frac{2}{3}$ n CaCl$_2$	49 da.	30 da.

[1] This occurred in only one case. The usual length of life was 48 hours.

In Loeb's experiments on Fundulus the most favorable results
were obtained when to Na both Ca and K were added in small
quantities. I find in Trout that, as a rule, the addition of K has no
effect. This is in accord with the suggestion made by Loeb that K
is indifferent so far as Na is concerned.[1] I have found, however, in
a few cases that when Ca is used in small quantities the addition of
K is distinctly advantageous; for instance, one specimen lived for
twenty days in the solution 100 c.c. $\frac{1}{2}$ n NaCl + 2 c.c. $\frac{2}{3}$ n CaCl$_2$ +
2 c.c. $\frac{1}{4}$ n KCl, twelve days longer than those used for control lived
in 100 c.c. $\frac{1}{2}$ n NaCl + 2 c.c. $\frac{2}{3}$ n CaCl$_2$, and fourteen days longer than
those in $\frac{1}{2}$ n NaCl. If Ca is used in large quantities, K is of no
advantage, for if an amount sufficient to counteract the Ca is used it

[1] LOEB, J.: Archiv für die gesammte Physiologie. 1900. lxxx, p. 229.

will cause the heart to stop beating. It is difficult to give a fair impression of such experiments by using averages, for individual differences often cause marked variation in results; for example, in a $\frac{2}{3}n$ NaCl solution the duration of life is, as a rule, about 24 hours; but in a few cases it lasted from 10 to 17 days. Also with the solutions 100 c.c. $\frac{5}{16}n$ NaCl + 12 c.c. $\frac{3}{8}n$ CaCl$_2$ (length of life 40 hours), and 100 c.c. $\frac{2}{3}n$ NaCl + 12 c.c. $\frac{1}{8}n$ CaCl$_2$ (length of life 23 hours), the specimens lived longer in the solution with the greater osmotic pressure, a result quite the contrary of that usually obtained. Again in the following experiment the addition of Ca seemed to have a harmful effect or a very slight favorable effect, whereas usually there was a difference of from one to seven days in favor of the calcium solution.

Solution.	Duration of life.	
100 c.c. $\frac{2}{3}n$ NaCl	18 hours	39 hours
100 c.c. $\frac{2}{3}n$ NaCl + 10 c.c. $\frac{5}{16}n$ CaCl$_2$	39 hours	42 hours
100 c.c. $\frac{2}{3}n$ NaCl + 12 c.c. $\frac{1}{8}n$ CaCl$_2$	23 hours	23 hours

These individual differences also caused difficulty in finding the optimum solution, that is, the solution containing the proportion of salts in which the specimens live and thrive best. The experiments seem to indicate that where three salts are used the optimum is 100 c.c. $\frac{1}{2}n$ NaCl + 2 c.c. $\frac{2}{3}n$ CaCl$_2$ + 2 c.c. $\frac{1}{4}n$ KCl; but where two salts are used the optimum is 100 c.c. $\frac{1}{2}n$ NaCl + 8 c.c. $\frac{2}{3}n$ CaCl$_2$, or 100 c.c. $\frac{2}{3}n$ NaCl + 12 c.c. $\frac{1}{8}n$ CaCl$_2$. The relative advantages of the three solutions may possibly depend upon the proportion of Ca and K in the tissues. If much Ca is present in the tissues, then little Ca is necessary in the solution, and the first mentioned solution would be better; if little Ca is present, then more is necessary in the solution, and either of the last two would be better. When the proportion of ions in the tissues and in the solution is properly balanced, a condition of tolerance may be established, — that is, a condition may be reached in which interchange of ions between the tissues and the solution no longer takes place. The specimens will then live indefinitely in the solution. It may be possible that a change in physical conditions in the colloidal substances of the cells is responsible for

the power of Ca to counteract the poisonous effects of Na. In a balanced solution of electrolytes, ions enter the tissues in such proportions that the relative amounts of solid and of liquid portions render the condition of the protoplasm favorable for enzyme action. The rhythmical activities depend upon this condition. Loeb has found that Na-, Ca-, and K-ions must exist in definite proportions in a tissue in order that it may contract rhythmically. " If the tissue has permanently or temporarily more Ca- and fewer Na-ions than are required for the above mentioned physical properties and condition of equilibrium, an increase of Na-ions in the tissue will cause rhythmical contraction. . . . If the tissue, however, contains too many Na- and too few Ca-ions, a further increase of the latter in the tissue will cause the beginning of rhythmical contractions." [1] The rhythmical contraction of the tail mentioned above was noticed in the following solutions : —

> 100 c.c. $\frac{2}{3}n$ NaCl.
> 100 c.c. $\frac{2}{3}n$ NaCl + 2 c.c. $\frac{1}{3}n$ NaCl₂.
> 100 c.c. $\frac{2}{3}n$ NaCl + 12 c.c. $\frac{1}{3}n$ NaCl₂.
> 100 c.c. $\frac{2}{3}n$ NaCl + 2 c.c. $\frac{2}{3}n$ KCl.
> 100 c.c. $\frac{2}{3}n$ NaCl + 2 c.c. $\frac{1}{3}n$ CaCl₂ + 2 c.c. $\frac{2}{3}n$ KCl.
> 100 c.c. $\frac{1}{3}n$ NaCl + 12 c.c. $\frac{2}{3}n$ CaCl₂ + 1 c.c. $\frac{1}{3}n$ KCl.
> 50 c.c. $\frac{2}{3}n$ NaCl + 50 c.c. sugar.

As a rule these contractions did not begin until some time after death. Specimens in which all normal activity had ceased in the evening were found the next morning with a twitching tail, and the phenomenon usually continued for several hours. After death the muscles on the uppermost side of the fish became contracted more than those on the other side ; the body was thus bent at right angles, the tail extending upwards. The contractions were therefore very noticeable. The fact that the tail is thin and can be longer supplied with oxygen than the other muscles, is probably favorable for the action of the ions present in the solution. To produce the contractions Na must enter the fish in sufficient quantities to cause death, and at the same time to form in the muscular tissue of the tail with the Ca and K present just that balanced proportion of ions which will insure a labile condition of the protoplasm and fit it for enzyme action.

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[1] Loeb, J.: This journal, 1900, iii, p. 394.

THE EFFECTS OF PURE SOLUTIONS OF OTHER ELECTROLYTES.

Na is not alone in its poisonous effects; any pure solution of an electrolyte will act as a poison. To show this, pure solutions of Ca, Mg, Li, K, were used. The results are given in Table II. That $\frac{2}{3}n$ LiCl allows life to continue for 24 hours is remarkable. K is harmful, because it is a specific poison for muscles, that is, muscles placed in a pure solution of K soon lose their power of contracting with comparative rapidity. It therefore causes the heart to stop beating as soon as it enters the tissue.

$CaCl_2$ was used with each of these salts to determine its antagonizing effects. It was found to be very efficacious in the case of Mg, and efficacious to a certain degree in the case of Li and K. In Trout the addition of Ca to K seemed to have no effect, but in tadpoles there was a decided difference. Further experiments with Trout might have shown the same results.

TABLE II.
Brook Trout.

Pure solutions.	Duration of life.		Solutions with $CaCl_2$.	Duration of life.	
	Max.	Av.		Max.	Av.
100 c.c. $\frac{2}{3}n$ MgCl$_2$	8 da.	2.5 da.	100 c.c. $\frac{2}{3}n$ MgCl$_2$ + 12 c.c. $\frac{1}{2}n$ CaCl$_2$	30 da.	4 da.
100 c.c. $\frac{2}{3}n$ LiCl	24 hrs.	16 hrs.	100 c.c. $\frac{2}{3}n$ LiCl + 12 c.c. $\frac{1}{2}n$ CaCl$_2$	2 da.	24 hrs
100 c.c. $\frac{2}{3}n$ CaCl$_2$	17 da.[1]	10 da.	100 c.c. $\frac{2}{3}n$ CaCl$_2$ + 1 to 10 c.c. $\frac{1}{2}n$ KCl	10 da.	7 da.
100 c.c. $\frac{1}{2}n$ KCl	3 da.	1.5 da.	100 c.c. $\frac{1}{2}n$ KCl + 10 c.c. $\frac{2}{3}n$ CaCl$_2$	3 da.	1.5 da.

[1] I should like to call attention to this rather remarkable result with CaCl$_2$. Unfortunately, I was unable to repeat the experiment, owing to a difficulty in obtaining a fresh supply of material.

In order to show that in these experiments the cation and not the anion was responsible, I tried the bromides of Ca, Na, and K with results no different from those obtained with the chlorides. In this connection Professor Loeb has asked me to mention an observation made by him, but not yet published. As the use of three chlorides together lessens the degree of dissociation of the NaCl, he combined NaBr and the two chlorides, KCl and CaCl$_2$. The results were not different.

The Effect of Non-Electrolytes.

The fact that the addition of $CaCl_2$ to NaCl causes an increase in the length of life might be interpreted to mean that Ca salts are necessary for life and that consequently life is lengthened when these are supplied. To test this point sugar solutions were used to which Ca in varying proportions was added. As sugar solutions are incapable of being dissociated, any ill effects from a pure sugar solution must be due either to osmotic pressure or to a specific poisonous effect, not to ionic effects. Loeb has called attention to the fact that sugar is not an indifferent substance. A $\frac{1}{4}n$ sugar solution has very nearly the same osmotic pressure as a $\frac{2}{8}n$ NaCl solution; a com-

TABLE III.

Brook Trout.

Sugar solutions + $CaCl_2$.	Av. dura- tion of life.	NaCl Solutions + $CaCl_2$.	Av. dura- tion of life.
100 c.c. $\frac{1}{4}n$ sugar	46 hrs.	100 c.c. $\frac{2}{8}n$ NaCl	45 hrs.
100 c.c. $\frac{1}{4}n$ sugar + 2 c.c. n $CaCl_2$	41 hrs.	100 c.c. $\frac{2}{8}n$ NaCl + 2 c.c. $\frac{1}{4}n$ $CaCl_2$	7 da.
100 c.c. $\frac{1}{4}n$ sugar + 5 c.c. n $CaCl_2$	36 hrs.	100 c.c. $\frac{2}{8}n$ NaCl + 5 c.c. $\frac{1}{4}n$ $CaCl_2$	4 da.
100 c.c. $\frac{1}{4}n$ sugar + 8 c.c. n $CaCl_2$	28 hrs.	100 c.c. $\frac{2}{8}n$ NaCl + 8 c.c. $\frac{1}{4}n$ $CaCl_2$	8 da.
100 c.c. $\frac{1}{4}n$ sugar + 12 c.c. n $CaCl_2$	27 hrs	100 c.c. $\frac{2}{8}n$ NaCl + 12 c.c. $\frac{1}{4}n$ $CaCl_2$	3 da.

parison of results must therefore be made on this basis. In a $\frac{1}{4}n$ sugar solution the maximum length of life was three days, and the average length 46 hours. In $\frac{3}{8}n$ and $\frac{2}{8}n$ sugar solutions, life was indefinite, lasting in several cases about 19 days. There was very little difference then between the effects of these solutions and those of the corresponding NaCl solutions. In solutions of greater concentration there was a marked difference. In $\frac{1}{4}n$ NaCl life never exceeded 2.5 hours while in n sugar life might last 27 hours. When $CaCl_2$ was added to a sugar solution it was found that, in general, the greater the amount added the sooner death ensues. The reverse of this occurred when $CaCl_2$ was added to NaCl solutions. This result is very striking when comparison is made in the same series. It indicates that something besides osmosis must account for the phenomena. (Table III.)

Possibly the strongest proof that no one ion is necessary for life is furnished by the fact that the specimens live so long in distilled water. The experiments indicate that animal tissues are not adapted to a single ion; the presence of one ion necessitates the existence of other ions in definite proportions to counteract ill effects and balance the solution.

EXPERIMENTS ON TADPOLES.

The same method was used for tadpoles, except that they were kept at the ordinary room temperature of about 68° F. Only those

TABLE IV.

Tadpoles.

Pure solutions.	Duration of life.		Solutions with CaCl₂.	Duration of life.	
	Max.	Av.		Max.	Av.
100 c.c. ⅔ n NaCl	1 hr.				
100 c.c. ⅓ n NaCl	9 da.	3.25 da.	100 c.c. ⅓ n NaCl + 2 to 12 c.c. ⅔ n CaCl₂	17 da.	7 da.
100 c.c. ⅔ n CaCl₂	30 hrs.	12 hrs.	100 c.c. ⅔ n CaCl₂ + 1 to 10 c.c. ⅓ n KCl	2 da.	19 hrs.
100 c.c. ⅓ n KCl	22 hrs.	10 hrs.	100 c.c. ⅓ n KCl + 10 c.c. ⅔ n CaCl₂	3.5 da.	2 da.
100 c.c. ⅓ n LiCl	27 hrs.	19 hrs.	100 c.c. ⅓ n LiCl + 12 c.c. ⅔ n CaCl₂	2 da.	1 da.
100 c.c. ⅓ n MgCl₂	8 da.	2.5 da.	100 c.c. ⅓ n MgCl₂ + 12 c.c. ⅔ n CaCl₂	12 da.	7 da.

that had passed the gill stage were used, and during the course of an experiment they were not fed. Approaching death was indicated by the appearance of white, opaque spots in the thinner part of the tail, marking dead tissue. In some solutions, notably NaCl and sugar, a tonic contraction of the tail took place, bending it at right angles to the long axis of the body or curling it around the body. No rhythmical contractions, however, were noted. The activities were suspended in the same order as in Trout, but since it was more difficult to tell just when respiration and the heart-beat ceased, loss of motion was with them taken as the mark of death. *In distilled water the heart sometimes beat as long as two days after motion of the body ceased.* In the salts, however, the interval was very much shorter, and disintegration soon followed cessation of the heart-beat.

The results obtained were uniform with those obtained from Trout, with the exception that tadpoles are somewhat more sensitive to salt solutions. $\frac{1}{4}n$ NaCl is as harmful for them as $\frac{2}{3}n$ NaCl for Trout. They are also more sensitive to an increase in the osmotic pressure of the solution, — sudden decrease in the length of life takes place as the strength of the NaCl solution is increased. Life in distilled water is indefinite. The sugar solutions used were made exactly isosmotic with the parallel NaCl solutions. The addition of Ca caused a decrease in the length of life when added to sugar, an increase when added to NaCl, as was the case with the approximately isosmotic solutions used for Trout. (Table IV.)

In most of the solutions noticeable shrinkage took place. This occurred with tadpoles usually as death approached. In distilled water, however, it was very marked some days before death. This confirms Lillie's[1] observation of the shrinkage of fresh water Planaria in distilled water. I do not find in tadpoles a return to the more primitive shape as noted by Lillie. The bulging body remains and bears the same size relation to the tail as before. In salt solutions shrinkage might seem due to the giving off of water, but the fact that it occurs in a more marked degree in distilled water speaks against this assumption, and shows that osmotic pressure is not sufficient to account for the effect of solutions upon living organisms. Possibly the shrinkage is due to a lack of food, as Lillie suggests in the case of Planaria. An observation made on Trout, however, does not support this theory. Certain specimens placed in $\frac{1}{4}n$ NaCl and in distilled water reached a stage in two weeks which those in tap-water did not reach for from four to six weeks. The yolk sac was almost completely absorbed. This could not have been due to lack of food. It may have been due to changes in temperature, or, as seems more probable, to the formation in the yolk sac of compounds which are absorbed more quickly than those ordinarily present. If compounds were formed which were less soluble, development might be retarded. This would be an interesting point to determine. Loeb has found that by changing ions in the tissues their power of absorbing water may be changed. If a muscle is placed in a solution of $\frac{1}{4}n$ CaCl$_2$ it loses about 20 per cent of its weight of water. In an equimolecular solution of KCl it absorbs about 50 per cent, in NaCl it absorbs none or very little.

[1] LILLIE: The American Naturalist. 1900. xxxiv. p. 173.

It may be remembered that Ringer [1] also has tried the effects of salts upon fish and tadpoles. His experiments were, however, conducted on a different basis. He believed distilled water to be a poison and attempted to neutralize its ill effects. Locke [2] has shown that the poisonous effects of the water used by Ringer were due to the fact that it contained heavy metal compounds. Ringer merely found that the addition of certain salts to this water would antagonize these compounds. As a very small amount was sufficient to do this, his solutions were very weak. He used a solution of NaCl 0.0004 n, where I used 0.25 n, and a solution of $CaCl_2$ 0.032 n where I used 0.5 n. His results were also vitiated by the fact that his dishes were left open to the air, so that a growth of micro-organisms was present. Concerning his interpretation of facts it is difficult to speak, for his opinions are tentative, and in his successive papers more or less contradictory. He deserves great credit, however, for being the first to recognize the antagonistic relations existing between Ca, Na, and K, and the advantage to be gained from combining them.

SUMMARY.

1. Pure solutions of the chlorides of Na, Ca, K, Mg, Li are poisonous.

2. The poisonous effects of a pure NaCl solution may be antagonized by Ca.

3. Ca is not necessary in itself, for it renders a sugar solution more harmful.

4. K does not antagonize the ill effects of Na, but it may antagonize Ca used in small quantities.

5. In weak solutions, sugar is as poisonous as isosmotic solutions of NaCl; in stronger solutions, not so poisonous.

6. The optimum solutions were found to be:

$$100 \text{ c.c. } \tfrac{1}{2} n \text{ NaCl} + 2 \text{ c.c. } \tfrac{2}{3} n \text{ CaCl}_2 + 2 \text{ c.c. } \tfrac{1}{2} n \text{ KCl.}$$
$$100 \text{ c.c. } \tfrac{1}{2} n \text{ NaCl} + 8 \text{ c.c. } \tfrac{2}{3} n \text{ CaCl}_2.$$
$$100 \text{ c.c. } \tfrac{2}{3} n \text{ NaCl} + 12 \text{ c.c. } \tfrac{1}{2} n \text{ CaCl}_2.$$

7. Salts are not directly necessary for the life of young Trout or tadpoles, for they live indefinitely in distilled water. If salts are

[1] RINGER: Journal of physiology, 1883, iv, p. 6; 1884, v, p. 98; 1885, vi, p. 154; 1890, xi, p. 79; 1895, xvii, p. 423.

[2] LOCKE: Journal of physiology, 1895, xviii, p. 319.

present, however, the metal ions Na and Ca must exist in balanced proportion.

8. In Trout respiration often stopped two or three hours before the heart-beat ceased; in tadpoles the heart sometimes beat two days after motion of the body ceased.

I should like to express my thanks to Professor Loeb for his kindness in giving me the assistance necessary for me to carry on the work. I wish also to acknowledge the courtesy of Mr. Arthur Sykes, through whom I was enabled to obtain material from the Fish-Hatchery at Madison, Wis.

THE INFLUENCES OF DIGESTION ON ANIMAL HEAT PROCESSES.

By EDWARD T. REICHERT.

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THE rise of body temperature observed during the periods of digestion was long since noted, and that this phenomenon is attributed to an increase of heat production is what should be anticipated because of the almost universal, although erroneous, belief that changes in body temperature and heat production are concomitants, the former depending upon the latter. There are, however, other reasons which warrant the view that "digestion fever" is caused by increased thermogenesis. For instance, Fredericq[1] found in experiments upon the human being that during digestion the absorption of oxygen may be increased as much as 45 per cent, and Langlois's[2] results of calorimetrical studies on children show that the curves of oxygen consumption and heat production are closely related. Laulanié[3] records in experiments on dogs that digestion increases heat dissipation, the consumption of oxygen, and the formation of carbon dioxide, and that the changes are in relation to the quantity of food. Finally, Rubner's[4] results prove that heat production and body temperature in dogs are higher during abundant feeding than during fasting, and, moreover, that the percentage increase of heat production is influenced to an important degree by the character of the diet.

The main object sought in the present research, which embodies ten six-hour experiments upon dogs, was the determination of the

[1] FREDERICQ: Archives de biologie, 1883. iv, p. 731.

[2] LANGLOIS: Journal d'anatomie et de physiologie, 1887, xxiii, p. 430

[3] LAULANIÉ: Comptes rendus de la société de biologie, 1892, xliv, p. 10

[4] RUBNER: Sitzungsberichte der Königlichen bayrischen Academie der Wissenschaften, 1895, Heft 4. Also Jahresbericht über die Fortschritte der Thier-Chemie, 1885, xv, p. 367.

influences of feeding upon the heat processes during the earlier
hours of the periods of digestion, that is, during the first four hours
after feeding, when the mean digestive activity in the dog is probably
at its maximum. The experiments were carried out by the aid of
the author's water calorimeter. The animals fasted at least eighteen
hours before the beginning of the experiments, and were studied for
two consecutive hours in the calorimeter before feeding, and for four
consecutive hours (with one exception for three hours) subsequent
thereto. In three experiments the diet consisted of flesh; in four,
of suet; in one, of beef-fat; and in two, of flesh and fat. The follow-
ing are the condensed records : —

Experiment 1. — Dog. Weight, 10.431 kilos. Fed 0.225 kilo of *flesh* at 39° at end
of second hour.

	Hourly heat produc- tion.	Hourly heat dissipa- tion.	Rectal temperature.			Mean room tempera- ture.
			Begin- ning of hour.	Ending of hour.	Gain (+) or loss (-).	
1st hour before feeding	20.002	22.505	39.55	39.25	− 0.30	22.7
2d hour before feeding	20.793	22.200	39.25	39.08	− 0.17	23.2
1st hour after feeding	20.883	21.991	39.08	38.95	− 0.13	22.7
2d hour after feeding	21.559	22.156	38.95	38.88	− 0.07	22.5
3d hour after feeding	20.071	19.900	38.88	38.90	+ 0.02	22.3
4th hour after feeding	20.917	21.770	38.90	38.80	− 0.10	23.2

Experiment 2. — Dog. Weight, 14.059 kilos. Fed 0.25 kilo of *flesh* at 38° at end
of second hour.

1st hour before feeding	26.336	28.473	38.42	38.23	− 0.19	21.9
2d hour before feeding	26.473	28.160	38.23	38.08	− 0.15	21.7
1st hour after feeding	33.660	30.326	38.08	38.38	+ 0.30	21.9
2d hour after feeding	25.063	25.400	38.38	38.35	− 0.03	22.2
3d hour after feeding	26.808	24.862	38.35	38.52	+ 0.17	21.8
4th hour after feeding	31.933	29.300	38.52	38.75	+ 0.23	21.6

Experiment 3. — Dog. Weight, 13.038 kilos. Fed 0.25 kilo of *flesh* at 39° at end of second hour.

	Hourly heat production.	Hourly heat dissipation.	Rectal temperature.			Mean room temperature.
			Beginning of hour.	Ending of hour.	Gain (+) or loss (-).	
1st hour before feeding	23.116	27.278	39.46	39.06	− 0.40	19.7
2d hour before feeding	23.785	25.871	39.06	38.86	− 0.20	19.8
1st hour after feeding	23.599	25.725	38.86	38.66	− 0.20	20.0
2d hour after feeding	26.041	23.596	38.65	38.88	+ 0.23	20.5
3d hour after feeding	27.605	25.054	38.88	39.12	+ 0.24	20.7
4th hour after feeding	24.002	27.414	39.12	38.80	− 0.32	21.3

Experiment 4. — Dog. Weight, 13.152 kilos. Fed 0.225 kilo of *suet* at 39° at end of second hour.

1st hour before feeding	23.487	24.539	38.85	38.75	− 0.10	21.1
2d hour before feeding	25.250	25.250	38.75	38.75	± 0.00	22.4
1st hour after feeding	25.781	25.781	38.75	38.75	± 0.00	21.8
2d hour after feeding	25.935	27.112	38.75	38.64	− 0.11	21.9
3d hour after feeding	28.069	25.500	38.64	38.88	+ 0.24	22.3
4th hour after feeding	24.469	25.967	38.88	38.74	− 0.14	21.9

Experiment 5. — Dog. Weight, 13.719 kilos. Fed 0.25 kilo of *suet* at 39° at end of second hour.

1st hour before feeding	25.905	28.649	39.13	38.68	− 0.45	20.0
2d hour before feeding	25.654	27.849	38.68	38.48	− 0.20	20.6
1st hour after feeding	27.646	26.549	38.48	38.58	+ 0.10	20.8
2d hour after feeding	26.936	26.154	38.58	38.65	+ 0.07	20.9
3d hour after feeding	27.415	26.318	38.65	38.75	+ 0.10	22.2
4th hour after feeding	24.600	24.600	38.75	38.75	± 0.00	21.7

Experiment 6. — Dog.   Weight, 9.524 kilos.   Fed 0.283 kilo of *suet* at 39° at end of second hour.

	Hourly heat production.	Hourly heat dissipation.	Rectal temperature.			Mean room temperature.
			Beginning of hour.	Ending of hour.	Gain (+) or loss (−)	
1st hour before feeding	24.159	25.378	39.08	38.92	− 0.16	19.0
2d hour before feeding	26.853	25.794	38.92	39.06	+ 0.14	19.2
1st hour after feeding	28.514	28.208	39.06	39.45	+ 0.39	19.4
2d hour after feeding	26.704	26.978	39.45	39.67	+ 0.22	19.4
3d hour after feeding	26.638	26.638	39.67	39.67	± 0.00	19.7
4th hour after feeding	28.548	27.920	39.67	39.75	+ 0.08	20.2

Experiment 7. — Dog.   Weight, 12.937 kilos.   Fed 0.25 kilo of *suet* at end of second hour.

	Hourly heat production.	Hourly heat dissipation.	Beginning of hour.	Ending of hour.	Gain (+) or loss (−)	Mean room temperature.
1st hour before feeding	26.278	29.900	38.95	38.60	− 0.35	19.8
2d hour before feeding	25.150	26.909	38.60	38.43	− 0.17	20.3
1st hour after feeding	26.850	23.580	38.43	38.74	+ 0.31	20.2
2d hour after feeding	26.096	24.830	38.74	38.86	+ 0.12	20.3
3d hour after feeding	22.956	22.956	38.86	38.86	± 0.00	21.9
4th hour after feeding	23.695	24.011	38.86	38.83	− 0.03	22.3

Experiment 8. — Dog.   Weight, 12.585 kilos.   Fed 0.225 kilo of *flesh* and *fat* at 39° at end of second hour.

	Hourly heat production.	Hourly heat dissipation.	Beginning of hour.	Ending of hour.	Gain (+) or loss (−)	Mean room temperature.
1st hour before feeding	19.125	22.347	38.70	38.38	− 0.32	18.3
2d hour before feeding	19.963	22.278	38.38	38.15	− 0.23	18.8
1st hour after feeding	26.007	22.930	38.15	38.45	+ 0.30	19.3
2d hour after feeding	22.979	23.286	38.45	38.42	− 0.03	20.1
3d hour after feeding	25.923	26.333	38.42	38.38	− 0.04	22.0
4th hour after feeding	25.295	22.733	38.38	38.63	+ 0.25	22.4

Experiment 9. — Dog. Weight, 8.617 kilos. Fed 0.453 kilo of *flesh* and *fat* at 39° at end of second hour.

	Hourly heat produc- tion.	Hourly heat dissipa- tion.	Rectal temperature.			Mean room tempera- ture.
			Begin- ning of hour.	Ending of hour.	Gain (+) or loss (—).	
1st hour before feeding	21.879	20.500	38.45	38.65	+ 0.20	22.3
2d hour before feeding	26.323	26.068	38.65	38.60	0.05	22.5
1st hour after feeding	28.280	27.554	38.60	38.70	+ 0.10	22.4
2d hour after feeding	25.564	23.750	38.70	38.95	+ 0.25	22.5
3d hour after feeding	26.592	25.648	38.95	39.08	+ 0.13	22.1
4th hour after feeding	25.645	25.500	39.08	39.10	+ 0.02	22.2

Experiment 10. — Dog. Weight, 13.605 kilos. Fed 0.25 kilo of beef *fat* at 39° at end of second hour.

1st hour before feeding	31.340	32.537	38.73	38.62	— 0.11	22.5
2d hour before feeding	35.576	34.488	38.62	38.72	+ 0.10	23.6
1st hour after feeding	38.629	37.944	38.72	38.78	+ 0.06	24.0
2d hour after feeding	35.991	38.276	38.78	38.57	— 0.21	26.1
3d hour after feeding	40.256	40.256	38.57	38.57	+ 0.00	25.4

A critical study of the foregoing records can more advantageously be made by considering the results as a whole than by regarding each experiment as a distinct unit, on account of the unstable char- acter of the heat processes, even in normal animals. In normal fast- ing animals I have shown[1] that well marked variations occur in heat production, heat dissipation, and body temperature from hour to hour, and generally without apparent cause. These variations are of so uncertain a character that typical effects can best be determined by considering collectively the results of a number of experiments carried out under as nearly as possible identical conditions. Thus,

[1] REICHERT: University medical magazine, 1890, iii. p. 345.

in normal fasting animals *composite curves* of heat production, heat
dissipation, and body temperature constructed from the results of six
six-hour experiments, show that when a dog is placed in a water
calorimeter, there is a distinct tendency for heat production, heat
dissipation, and body temperature to fall continuously during the
entire six hours, rapidly at first, and in constantly decreasing ratio
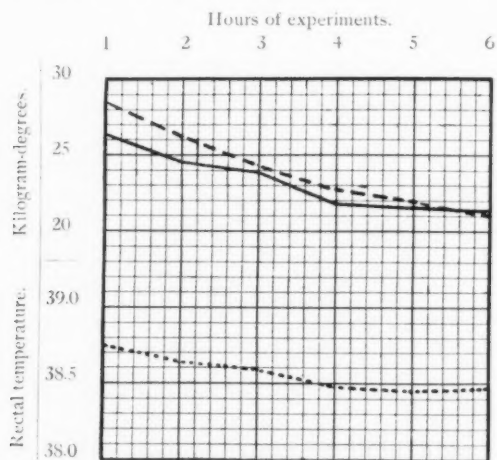from hour to hour (Fig. 1).

FIGURE 1. — Curves of heat production (———), heat dissipation (— — — —), and body
temperature (......) in normal fasting animals.

Such curves serve as an important basis for comparison of the
results obtained under other conditions. If now similar *composite
curves* be constructed of the records of the experiments embodied in
the present paper, any important differences will be rendered mani-
fest (Fig. 2).

Comparing first the composite curve of heat production of fasting
animals with that of animals before and after feeding, it will be
observed in the former that heat production falls during the entire
six hours ; and in the latter, that there occurs a well-marked increase
after the ingestion of food, that this increase is most marked dur-
ing the first hour after feeding, and that heat production continues
increased through the entire four hours.

The curves of heat dissipation are also strikingly unlike. In fast-
ing animals the curve of heat dissipation remains continually above

that of heat production, excepting during the sixth hour, and falls
steadily during the six hours. In animals after feeding it is continu-
ally below that of heat production, and remains at about the same
level throughout the experiments.

In fasting animals heat production is less than heat dissipation,
causing a fall of temperature; during the periods of digestion, heat
production is greater than heat dissipation, causing a rise of temper-
ature. The curves of body temperature also are dissimilar, there
being a well-defined downward tendency in fasting animals, and the
reverse, but more marked, in animals after feeding. During diges-
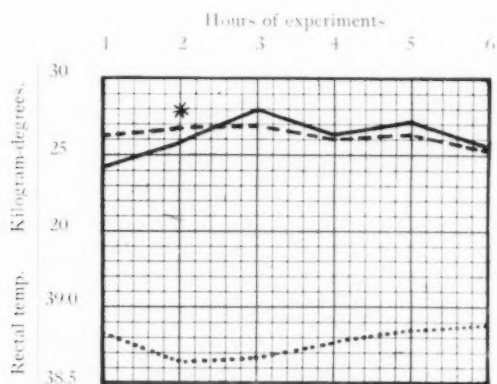
FIGURE 2. — Curves of heat production (————), heat dissipation (— — — —) and body
temperature during digestion (.......). The asterisk marks the time of feeding.

tion body temperature rose continuously, reaching a maximum
increase during the fourth hour. The changes in body temperature
are not, however, proportional to those in heat production. In fact,
the greatest increase of heat production was observed during the first
hour after feeding, and the least increase during the fourth hour,
while the lowest temperature was recorded during the first hour, and
the highest during the fourth hour. The increase of temperature
was accompanied throughout by an increase of heat production.

The maximum increase of heat production was observed during
the first hour after feeding, and amounted to 2.185 calories, or nearly
nine per cent above the heat produced during the preceding hour.
The mean increase of heat production during the four hours of
digestion was 4.3 per cent. The mean increase of heat production

after feeding flesh was 5.7 per cent, after suet and beef-fat, 2.6 per cent, and after flesh and fat, 7.3 per cent. The maximum increase of temperature was noted during the fourth hour after feeding, amounting to 0.24° C. above the temperature recorded at the time of feeding.

In a research on dogs when fasting and well-fed, Rubner recorded a maximum difference in temperature of 0.3° C., and that a diet of flesh increased heat production 19.7 per cent, and a diet of fat, 6.8 per cent. Rubner's results and my own are in accord; the differences are quantitive, and are mainly, if not solely, owing to the different conditions under which the experiments were conducted.

The chief conclusions justified by the records of the above experiments are: (1) that the rise of temperature observed during the period of digestion is due to an increase of heat production; (2) that the temperature gradually rises and reaches a maximum during the fourth hour, or possibly later; (3) that the greatest increase of heat production occurs during the first hour after feeding; (4) that the changes in temperature and heat production are not proportional; (5) that the most marked effects, as a whole, are observed when the diet consists of proteid and fat, next with proteid, and least with fat; (6) that the increase of heat production is not nearly so great as is indicated by the results of the oxygen experiments of Fredericq.

REACTION OF ENTOMOSTRACA TO STIMULATION
BY LIGHT. — II. REACTIONS OF DAPHNIA
AND CYPRIS.

By ROBERT M. YERKES.

CONTENTS.

IN the first paper of this series (Yerkes, 1899)[1] the results of an
experimental study of the reactions of *Simocephalus vetulus*
Mueller to differences of light intensity, and also to different colors,
was given. When that work was in progress, Daphnia, a form which
I wished to test, was not available; but during the past year I have

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[1] YERKES: This journal, 1899, iii, pp. 157–182. The complete bibliographic
references to papers cited will be found at the end of this paper, alphabetically
arranged, under the heading "Bibliography."

been able to obtain the desired material, and this paper deals with the reactions of *Daphnia pulex* DeGeer, as a representative of the Phyllopoda, and of *Cypris virens* Jurine, an Ostracod.

I. STATEMENT OF PROBLEMS.

Briefly stated, the objects of the experiments to be described were as follows:

1. To study comparatively the relation of rate of movement to intensity of light in animals of two orders, the Phyllopoda and the Ostracoda.

2. To discover whether the reversal of reaction which Towle (1900)[1] has described for Cypridopsis occurs in other forms, and if so by what it is caused.

3. To determine the influence of other stimuli, *e. g.* heat and chemicals, on the reactions to stimulation by light.

4. To test further by a new method the *so-called* photopathic reactions of certain Crustacea.[2]

II. ANIMALS EXPERIMENTED WITH AND METHODS OF COLLECTING.

During the fall of 1898 Simocephalus vetulus, a Cladoceran very similar to Daphnia pulex, existed in abundance in several small po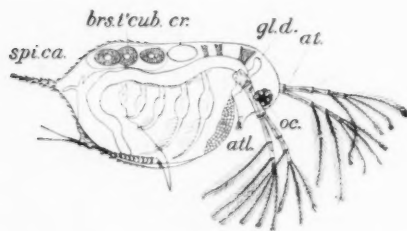nds in Cambridge, but Daphnia were scarce. Last fall the reverse was true. Daphnia pulex (Fig. 1) swarmed in certain places, while Simocephalus was found in small numbers.

The material for the following work, both Daphnia and Cypris, was obtained in a small rain-water pond that presumably is dry during the summer. Here Daphnia could be seen in large numbers on the sunny side of the pond and Cypris virens (Fig. 2) abounded in the grass and mud of the bottom.

FIGURE 1. — Daphnia pulex DeGeer. × 13. *at.* antenna; *atl.* antennule; *gl. d.* dorsal gland; *cr.* heart; *brs. t'cub.* brood pouch; *spi. ca.* caudal spine; *oc.* eye.

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[1] TOWLE: This journal, 1900, iii, p. 352.

[2] An account of this work (4) will be given in the next paper of this series.

By means of Birge's (1881-91, pp. 397-398)[1] Cone Dredge it was possible in a few minutes to collect thousands of the animals. I wish to take this opportunity to call attention to Birge's ingenious device, because it is very convenient for this kind of work and a valuable time saver. The apparatus, of which Fig. 3 is a sketch, consists of a wire cone of two-millimetre

FIGURE 2. — Cypris virens Jurine. × 17. *at.* antenna; *atl.* antennule; *pd.₁* first foot.

mesh, C, through which the animals to be collected may easily pass. To opposite sides of this cone is soldered a strong wire, which is bent into a ring, R, at the apex of the cone for the attachment of a cord with which to draw the dredge through the water. The base of the wire cone is soldered to a metal band, to which is fastened one end of a fine cloth net, N. The other and narrower end of the net is fastened to a metal flange which carries a screw cap, S. As the dredge is drawn through the water small animals pass through the meshes of C and are carried back into S. When the collecting is completed, the cap is unscrewed and its contents emptied into a collecting vessel. In this way the desired material is obtained easily, quickly, and free from dirt.
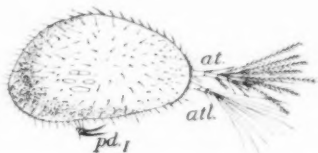
Fig. 3. — Birge's Cone Dredge. R. ring for attachment of line; C. wire cone; N. cloth net; S. screw cap.

III. RELATION OF RATE OF MOVEMENT TO INTENSITY OF LIGHT.

The problem stated in its simplest form is this, — Does there exist any definite relation between the rate at which an entomostracan moves and the intensity of light rays striking it?

Some years ago observations on this subject were made in this laboratory by Davenport and Cannon (1897, pp. 29-32),[2] which led to the conclusion that within certain limits rate of movement increases slightly with a considerable increase in intensity of light.

[1] BIRGE: Transactions of the Wisconsin academy of science, viii, pp 397-398.
[2] DAVENPORT and CANNON: Journal of physiology, 1896. xxi, pp. 29-32.

This result was reached through a study of Daphnia, an extremely sensitive form; I have worked with Cypris, which is far less positively heliotropic, but have also used Daphnia for the sake of comparisons. With Daphnia, Davenport and Cannon found that the time required to move 16 cm. increased 18 per cent when the intensity of the light was decreased to one-fourth of its former intensity.

Time in " full light." . . . . 35.7 sec.
Time in " one-fourth light." . . . . 41.2 sec.
Ratio of time in " full light " to time in " one-fourth light," 87 : 100.

Method. — A tin trough, in reality a half cylinder with a radius of 8 cm., 60 cm. long, painted dead black inside and out, and mounted on a wooden base for convenience of movement, served for these experiments. The ends of the trough were glass, so that the light from a Welsbach burner could enter the trough parallel to its long axis.

To exclude the influence of heat rays a vessel containing alum solution was placed at the end of the trough through which the light entered. The opposite end and sides, except where observations were being made, were screened from reflected light by pieces of black cardboard.

Different intensities of light were obtained by moving the burner instead of diminishing the quantity of gas. For the first series of observations to be described, the light was 75 cm. from the point at which the animals started.

FIGURE 4. — *x, y,* ends of trough; A, point at which animal started; C, point at which trip was finished; distance A C = 50 cm.; distance A B and B C each 25 cm.; distance of light from starting point (A D) 75 cm.

Fig. 4 indicates the arrangement for the first series. If we call the intensity of light at A 1, the intensity at B, which is 50 cm. instead of 75 cm. from the light, would be 2¼, and at C, 25 cm. from the light, 9.

For a second series the light was placed 150 cm. from the starting

point. In this case, since the distance from the burner to the starting point is just twice as great as in the first series, the intensity of the light at A is one-fourth as great, or $\frac{1}{4}$ instead of 1. The intensities at A, B, and C in the second series are then $\frac{1}{4}$, $\frac{3}{25}$, and $\frac{3}{16}$. Obviously the ratios of the intensities in the two series for particular points in the trough vary widely. Thus at A it is 4 : 1; at B it is $6\frac{1}{4}$: 1; at C it is 16 : 1.

An animal was gently dropped from a pipette into the trough at the negative (−) end, *i. e.*, the end farthest from the light. The instant it crossed a line marking the starting point A, 5 cm. from the − end, a stop-watch was started and a record made of the time taken for the passage from A to B and from B to C. Thus we have the periods for the first and second halves of the trip, and can at once compute the effect of intensity. If, as rarely happened, an animal, after crossing the starting point, returned to the − end, the experiment was discarded.

All observations were made on animals fresh from the pond at a temperature varying little from 20° C.

Experiments with Daphnia. — *a. In artificial light.* — Each series of experiments consists of a set of ten trials with each of five individuals. Before giving averages a representative set of experiments may be examined. Table I is a record of such a set.

For this set of experiments the first half of the distance took 1.8 seconds longer than the second half. The whole distance traversed was 50 cm. and the average time, 52.8 seconds. This Daphnia therefore moved at the rate of almost one centimetre per second.

Many of my sets, though not this one, show that in successive trips there is an increase in rate, a phenomenon to which Davenport and Cannon (1897, p. 32) have already called attention. After a Daphnia has made a few trips it is found to orient itself much more quickly and precisely than at first. This is very noticeable, even without measurements. I am at a loss for an explanation, unless it be due to a polarization of the animal which causes it to take a definite position in relation to the rays more quickly after the reflex movement, or complex of movements, called orientation has been repeated several times. But this of course is no explanation, and the problem remains an interesting one for analysis.

The results of five sets of observations similar to that of Table I are presented in Table II. For ease of comparison the average times

TABLE I.

Daphnia No. 2.   June 12, 1900, 10 A. M.   Temperature 20° C.
Distance of light at start, 75 cm.

Number of the experiment.	Time in seconds.		
	First half.	Second half.	Whole trip.
1	34	28	62
2	28	25	53
3	22	23	45
4	23	21	44
5	24	22	46
6	28	27	55
7	25	25	50
8	29	30	59
9	28	29	57
10	32	25	57
Averages	27.3	25.5	52.8

TABLE II.

Average times of trips in seconds for Daphnia pulex under the influence of different intensities of light.

Set.	First half. Distance of light.		Second half. Distance of light.		Whole trip. Distance of light.	
	75 cm.	150 cm.	75 cm.	150 cm.	75 cm.	150 cm.
1	26.8	44.0	27.4	47.7	54.2	91.7
2	27.3	36.9	25.5	38.0	52.8	74.9
3	44.3	46.0	37.1	38.7	81.4	84.7
4	36.6	43.2	30.3	35.3	66.9	78.5
5	31.4	37.9	27.7	31.9	59.1	69.8
Averages	33.28	41.60	29.60	38.32	62.88	79.92

for the light at 75 cm. distance and at 150 cm. have been arranged in adjoining columns.

Comparison of the *averages* of Table II furnishes the following ratios:

1. Ratio of First Half time at 75 cm. to same at 150 cm., 1 : 1.25. In other words, the time was one-fourth longer for the lower intensity of light; the intensities themselves being approximately in the ratio of 5.12 : 1.

2. Ratio of Second Half time at 75 cm. to same at 150 cm., 1 : 1.29.

3. Ratio of First Half time at 75 cm. to Second Half time at 75 cm., 1.12 : 1. The first half of the course, being less intensely illuminated, required one-eighth longer.

4. Ratio of First Half time at 150 cm. to Second Half time at 150 cm., 1.14 : 1.

5. Ratio of Whole Trip time at 75 cm. to same at 150 cm., 1 : 1.27.

An examination of these ratios shows at once what appears to be a very constant relation between intensity of light and rate of movement. In the case of the first ratio, for example, the relative intensities of the first half at 75 cm. and at 150 cm. are 5.12 : 1, and the times given are 1 and 1.25 respectively.

b. In Daylight. — One series of observations in daylight gave similar results. The same trough was used, being in this case directed toward an east window, from which diffuse light fell upon it.

<div align="center">RESULTS.</div>

Average Time for First Half	26.44 seconds.
Average Time for Second Half	23.48 seconds.
Average Time for Whole Trip	50.04 seconds.

Ratio of First Half time to Second Half time, 1.13 : 1. The first half took on an average 2.96 seconds longer than the second.

Experiments with Cypris. — *a. In artificial light.* — Experiments with Cypris were made by the same methods as with Daphnia. Table III is given to show the striking increase in rate throughout a set.

There is here only a very slight difference, 0.6 of a second, between the average times for the two halves.

Table IV gives the averages of five sets of experiments with Cypris, as Table II did for Daphnia. As before, each set gives the averages for ten observations on a single animal.

TABLE III.

Cypris No. 1. June 14, 1900, 2.30 P. M. Temperature 20° C.
Distance of light at start, 75 cm.

Number of the experiment.	Time in seconds.		
	First half.	Second half.	Whole trip.
1	28	29	57
2	27	31	58
3	33	22	55
4	17	16	33
5	16	19	35
6	16	19	35
7	17	15	32
8	15	14	29
9	16	16	32
10	16	14	30
Averages	20.1	19.5	39.6

TABLE IV.

Average times of trips in seconds for Cypris virens under the influence of different intensities of light.

Sets.	First half. Distance of light.		Second half. Distance of light.		Whole trip. Distance of light.	
	75 cm.	150 cm.	75 cm.	150 cm.	75 cm.	150 cm.
1	20.1	21.0	19.5	18.4	39.6	39.4
2	25.1	16.9	26.7	15.1	51.8	32.0
3	25.1	23.9	23.1	22.5	48.2	46.4
4	28.2	18.4	24.0	19.0	52.2	37.4
5	22.2	21.2	20.6	17.0	42.8	38.2
Averages	24.14	20.28	22.78	18.40	46.92	38.68

By making the same comparisons of averages for Cypris as were made for Daphnia (p. 411) the following ratios are obtained:

1. Ratio of First Half time at 75 cm. to same at 150 cm., 1.19 : 1; an increase in time accompanying an increase in intensity of light, a result contrary to that obtained with Daphnia.

2. Ratio of Second Half time at 75 cm. to same at 150 cm., 1.24 : 1; another case of increase of rate with decrease of intensity.

3. Ratio of First Half time at 75 cm. to Second Half time at 75 cm., 1.06 : 1. In this instance the result is similar to that given by Daphnia, but less marked, being 1.06 instead of 1.12.

4. Ratio of First Half time at 150 cm. to Second Half time at 150 cm., 1.10 : 1. This is also like the corresponding result with Daphnia, although slightly less.

5. Ratio of Whole Trip time at 75 cm. to same at 150 cm., 1.21 : 1.

Generally speaking, Cypris seems to show a less definite relation between rate and intensity than Daphnia. Cypris, however, moves much more rapidly and in a straighter course than Daphnia, and it is probable that there is with it less increase in rate with increased intensity of light simply because intensity affects rate of movement chiefly through rapidity and precision of *orientation*. Since Cypris, after having once oriented itself, takes a more direct path and moves more rapidly than Daphnia, the above results are not surprising.

b. In daylight. — In one set of experiments made in daylight, as in the case of Daphnia, Cypris reacted thus:

Average Time for First Half . . . . 22.40 Seconds.
Average Time for Second Half . . . . 20.60 Seconds.
Average Time for Whole Trip . . . . 43.00 Seconds.

Ratio of First Half time to Second Half time, 1.09 : 1. This is almost the same as for Daphnia.

Clearly the time taken to cover a certain distance depends upon (a) quickness and accuracy of orientation, (b) rapidity of swimming movements. That orientation increases in precision with increase in intensity of light up to a certain point is undoubtedly true; that the force and rapidity of an animal's swimming motions increase in like manner seems probable, and although the data thus far given do not prove it, I am prepared to say that both Daphnia and Cypris swim more quickly in strong than in weak light. Of this phenomenon

conclusive evidence was obtained in experiments soon to be described, in which the animals while moving in ordinary light were stimulated by a more intense light from above.

IV. REVERSAL OF REACTION.

For the Ostracod, Cypridopsis, a peculiar change in reaction has been described by Towle (1900, p. 352)[1]. An individual placed in a trough with a light at one end was found commonly to move toward the light. If, while it is so moving, and before it has reached the positive (+) end of the trough, the light is moved to the opposite end, the animal immediately turns and again swims toward the light, thus maintaining a positive heliotropic reaction; but if, after this has been several times repeated, the Cypridopsis is allowed to reach the positive end, it sooner or later turns and swims away from the light. If now, as before, the light is moved to the opposite end of the trough the animal turns in its course. By thus shifting the light from end to end this negative reaction may be kept up just as the positive one was. It thus appears that Cypridopsis may give either a positive or a negative reaction under conditions apparently the same.

Why this change in kind of reaction? Towle fails to find any explanation for it, and merely calls attention to the fact that the change from positive to negative "is brought about solely by internal causes, while the more immediate cause of the latter (*i. e.* the change from negative to positive) is an external one, namely, mechanical stimulation."

To determine whether a like change of reaction occurs in other Entomostraca, and also if possible to discover its causes, I have studied Daphnia and Cypris by methods similar to Towle's. The first objective point has been reached, but the second has been approached only in so far as negative results lead toward it.

Method. — A tin trough $8'' \times \frac{1}{2}'' \times \frac{1}{4}''$ (Fig. 5, T) mounted on a wooden base was painted dead black; at either end of this trough a glass box, A, A', containing alum solution was placed. Screens, S, S', were arranged so that side rays and reflected light were cut off, and the trough was illuminated exclusively by rays parallel with its long axis coming through holes six inches high and two inches wide cut in the screens S, S'. At either end, ten inches from S and S'

[1] *Loc. cit.*, p. 352.

respectively, was a Welsbach gas burner, L, L'. For observations this apparatus was set up in a dark room. After the trough had been filled with water and the screens s, s', which shut off all light, had been placed in position, an animal was carefully dropped into the middle of T. One of the screens (s) was then removed and the animal responded usually with a + reaction, — it moved toward the end from which the screen had been removed, that is toward the light. As soon as the animal came within two centimetres of the + end of the trough, s was quickly replaced and s' removed, thus giving light from the opposite direction without the inconvenience of moving the burner. By this means it could easily be observed whether the response was continued as before or reversed.
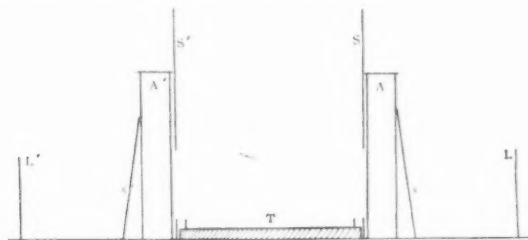

FIGURE 5. — Heliotropic apparatus, one-sixth actual size. T, tin trough; S, S', screens with rectangular openings for light; A, A', vessels of alum solution; s, s', cardboard screens; L, L', Welsbach burners.

In all these experiments a temperature of about 22° C. was maintained. In all cases the animals had been recently collected and were kept in shaded Stender dishes in the dark room. As determined rather roughly by the Bunsen photometer, the intensity of the light used was 22-candle power. The influence of heat rays was guarded against by the use of the adiathermal screens of alum solution already alluded to. Geotactic reactions were excluded by preliminary tests, as follows: the trough was set upon a table as nearly level as possible, and a series of observations made; it was then turned end for end, and a similar series made. Since all factors except gravity were constant, any tendency toward one end indicated a geotactic reaction, and was corrected by changing the position of the trough before further experimentation.

Reactions to Changes in Direction of Light. — *a. Daphnia.* — Daphnia is very strongly heliotropic and almost always gives a positive response at first. Observations on individuals were made in the fol-

lowing manner. An animal was taken up in a pipette of as large calibre as practicable, to avoid the influence of contact with the sides, and dropped into the middle of the experimenting trough, T; its reaction was noted; then an effort was made to keep up this first response by changing the direction of the light. Usually the Daphnia began with a positive reaction, and this could be continued indefinitely by changing the direction of the light.

Towle says that in Cypridopsis the change from a positive to a negative reaction occurs more readily than the reverse. The opposite is true of Daphnia pulex. This difference is probably due to Cypridopsis being either negatively heliotropic, or at least much less strongly positively heliotropic than Daphnia. With the majority of Daphnia either a + or − reaction can be maintained, but the + is much more definite, and lasts longer. In other words the + is the normal response.

As to the influence of contact with the inner surface of a pipette on the reactions of Daphnia, my observations do not agree with Towle's for Cypridopsis. She holds that taking up a − animal in the pipette will uniformly render it +. I was able in many cases to make + Daphnias − by taking them up several times, but this was not always true. In fact toward the end of my work I never succeeded in getting this result. Scores of times efforts were made to test the effect of the pipette on − animals, but the Daphnia were always so slightly − that this was useless. Even after having given several − reactions in response to changes in the direction of light, the animal upon being taken up and replaced in the trough would immediately become +. Although it may be argued that this was the result of contact with the sides of the pipette, the experiments were so unsatisfactory, because of the weakness of the negative tendency, that this conclusion does not seem justified. If animals could be found concerning whose − reaction there was no uncertainty, the question might easily be answered; but such animals I was unable to find, and I never succeeded in rendering a positive individual strongly negative.

b. Cypris. — Cypris gives reactions very similar to those of Daphnia. Briefly stated, they are as follows. Either a positive or a negative response may be maintained by changing the direction of the light; the positive, however, is more easily obtained and more lasting.

In no case was a + Cypris rendered − by the pipette. In this respect it differs from Daphnia. Negative animals were uniformly

made positive by the operation. In this, Cypris agrees with Cypridopsis. This result is an indication that change of reaction due to the use of the pipette is dependent upon the relative *strength* of the positive and the negative tendencies. In Cypris and Cypridopsis the positive tendency is much weaker than in Daphnia, hence the difference in the effect of the pipette.

Reactions to Changes in Temperature. — Thinking that perchance change of temperature might be the cause of the reversal of reaction which Towle describes, I tried experiments with Daphnia and Cypris to test this hypothesis. That changes in heliotropism are correlated with changes in temperature has been proved by Loeb (1893, p. 91)[1] for several animals. In the case of Polygordius larvæ, for example, at 24° C. all the animals are positively heliotropic, at 29° C. all are negative.

a. Daphnia. — To determine whether a similar change occurs in Daphnia, a porcelain dish 12 inches in diameter, so placed that diffuse daylight illuminated one side while the remainder of the dish was shaded, was filled with water at 22° C., containing several hundred Daphnia. Within a few minutes after being placed in the dish almost all of the animals collected in the illuminated region. The temperature was then gradually raised by the application of heat from below. At 28° there seemed to be a scattering of the animals, but there was no good evidence of a negative reaction to the light. At 32° all were positive except a few weaklings which were succumbing to the heat. Before 40° was reached all the Daphnia had perished.

b. Cypris. — By the same method Cypris gave similar results. There was absolutely no indication of a change of response. The only difference between Daphnia and Cypris in this respect is that Cypris can withstand a higher temperature. At 40° they survive for some time.

These experiments were repeated several times under different conditions of light, and there is, in my opinion, no doubt that such a modification of heliotropic reaction as Loeb found in Polygordius does not occur in these forms.

We are therefore forced to conclude that change of temperature is not at the bottom of the influence accompanying the use of the pipette.

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[1] LOEB: Archiv für die gesammte Physiologie, 1893, liv. p. 91.

Reflex Theory. — *a. Reactions to Sudden Changes in Intensity of Light.* — Thus far, as a possible factor in the peculiar reversal of response to light, we have considered change of temperature. *A priori* it seemed not improbable that even the slight difference in temperature arising from the use of a pipette in taking up the animals might influence the heliotropic reaction. For this supposition, however, the foregoing experiments furnish no support. The cause of the change in response, therefore, is yet to be found.

One of the first explanations to suggest itself was that there might be a polarization of the animal, *i. e.*, the passing of light rays through an individual in a given direction for a few minutes may be assumed so to modify it as to cause the organism to orient itself in respect to the direction of these rays. We can easily believe that light passing from anterior to posterior in an animal's body affects it differently from light passing in the opposite direction. This offers a verbal explanation for the maintenance of a definite position in respect to the direction of the light.

Another possibility lay in the domain of what may be termed a definite reflex. It may be that Daphnia, for instance, reverses its direction in response to *any* sudden stimulus, — that there is a reflex which determines the direction of movement. In such an event, it is clear that the light might simply call forth a certain complex of muscular contractions which would change the animal's position, and the *direction* of subsequent movement would thus be determined by the reflex. The direction of the light in itself therefore might not be of primary importance.

What is the reaction to the sudden stimulus given by light coming from directly above the animal? If a Daphnia is placed in a box so that it can move freely in any direction, but without having rays of light as important determinants of the direction of its movements, and a beam of strong light is thrown upon it from above in such a way that there is no reflection from the sides of the box, it is found that the animal continues its course uninterruptedly. My earliest observations seemed to indicate that there was a turning in response to this sudden stimulus, but further experiments showed this to be a reaction to reflected light. Similar observations were made for Cypris with like results. Neither Daphnia nor Cypris responds to a stimulus of this kind by turning; there is therefore no definite reflex which would explain the turning of an animal in response to change

of direction of light. In both forms a quickening of movement was very noticeable as a result of this sudden illumination from above.

b. Reactions to Acids. — To test further the reaction to sudden stimuli the result of contact with acids was studied. The $8'' \times \frac{1}{2}''$ $\times \frac{1}{4}''$ trough previously described was used. It was set in front of a window with its long axis either parallel to the window, or perpendicular to it. In the latter case the light of course tended to direct the animals toward the window, in the former the narrowness of the trough prevented such a reaction. I take the following record from my note-book.

May 12, 1900, 4.30 P.M. — An 8-inch trough was filled with water and placed perpendicular to the window. Into the end toward the window ten drops of strong HCl were put; into the opposite end, twenty Daphnia. The animals all moved toward the window (*i. e.* toward the source of light) and swam directly into the acid. On first reaching the acid zone they zigzagged about a little and increased their activity, but none turned back; in a few seconds all were dead.

This result is contrary to what would be true if all sudden and strong stimuli called forth a " turning reaction."

When the trough is turned parallel to the window there is a marked difference in the reaction. The animals swim to the edge of the acid, and while occasionally one enters it, the majority turn back and avoid the stimulating substance; the turning is not a quick, definite reflex by which the animal springs around, as it were, but a slow irregular and extremely variable turning. From this it is evident that the directive influence even of diffuse daylight is surprisingly strong, being sufficient to lead an animal to its destruction.

In 1 per cent chromic acid used similarly, with the trough per-

FIGURE 6.

pendicular to the window (see Fig. 6), the animals moved directly through the acid region, and as the acid was not strong enough to kill them immediately, they continued swimming about on the positive side of the acid, *i. e.* in the end of the trough nearest the window. After a time some began to move back toward the —

end, but as soon as they touched the acid zone they turned toward the window (+) again.

Ostracods (Cypris and two unidentified forms) usually stop swimming and sink to the bottom the instant they touch the acid zone. If not immediately killed, they swim ahead again after a few seconds. Their actions are in most respects very similar to Daphnia's. With them, too, light is a powerful directive agent.

Since from the results of these experiments the reflex theory does not seem tenable, we are driven back upon the polarization theory.

Polarization Theory. — Polarization in this case probably signifies a complex of factors of orientation which are as yet unknown except *en masse*. Supposing light has been striking an animal in a definite way, after a time the stimulus resulting from this particular sort of relation between light and organism will, it may be assumed, diminish. If the direction of the light now be changed, it is certain that a stronger stimulus will result, and we may well suppose that the animal will try to orient itself in its former relation to stimulating factors in the environment, so that there may be the least possible irritation. We are here making two assumptions which are possibly unwarrantable: that the organism is irritated by strong light, and that the strength of the stimulus, on repetition, must be increased to give the initial effect. But that these are warrantable assumptions seems highly probable from our knowledge of the influence of light on other animals. Granting these conditions, the animals would tend to keep constant the direction in which light strikes their bodies, and this is precisely what they for a time do.

Reactions of Simocephalus, and Towle's Criticism. — Towle (1900, p. 365)[1] quotes from my study of the reactions of Simocephalus the statement that three minutes was the time most favorable for a photopathic response, and that with a longer time there is a slight decrease in the number of animals in the + end of the trough. She criticises my interpretation of this fact in the light of her own work with Cypridopsis. I maintained that the lessening of the + reaction was due to the chance wandering of the animals; three minutes gave all time to orient themselves and move into the light region, but after a longer interval some of them had begun to wander back toward the — end. The wandering, Towle thinks, is probably due to the fact that the animals are rendered positive by being disturbed,

[1] *Loc. cit.*, p. 365.

and since this positive condition wears off in a short time, they then tend to go in the opposite direction.

In regard to this suggestion — for the criticism is only tentatively made — I wish to say that all my observations of Daphnia and Simocephalus indicate that the positive response does not disappear, that it is on the contrary extremely strong and uniform. Towle makes a mistake, I believe, in criticising results given by other forms in the light of studies on Cypridopsis. Although I have been unable to obtain Cypridopsis for my own study, I feel confident that it is much less strongly positively heliotropic than Daphnia.

This work was done in the Harvard Zoological Laboratory under the direction of Professor E. L. Mark, to whom I am indebted for aid and encouragement in experimentation and for a careful revision of this report. My thanks are also due Professor G. H. Parker for criticism and for his interest in the work, and to Professor C. A. Kofoid of the University of Illinois for the verification of my determination of the species of Ostracod used.

V. SUMMARY.

1. Daphnia pulex shows a marked increase in rate of movement with an increase in intensity of light.

2. This increase in rate depends chiefly upon precision and quickness of orientation, although there is also evidence of a quickening of the swimming motions.

3. Cypris virens exhibits a slight increase in rate with increase of intensity, but less uniformly than Daphnia.

4. The difference between Daphnia and Cypris in this respect is probably due to the greater importance for Daphnia of orientation as a factor in the determination of rate.

5. Either a positive or a negative reaction may be given by Daphnia for considerable periods. The negative reaction, however, is the less permanent. The same is true for Cypris.

6. Contact with the sides of a pipette appears to render a negative animal positive. This is undoubtedly true for Cypris, questionably so for Daphnia.

7. The heliotropic reactions of Daphnia and Cypris do not change with changes in temperature, so far as observed.

8. For these forms light is a sufficiently strong directive agent to lead them into fatal acid solutions.

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EXPERIMENTS ON ARTIFICIAL PARTHENOGENESIS IN ANNELIDS (CHÆTOPTERUS) AND THE NATURE OF THE PROCESS OF FERTILIZATION.

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CONTENTS.

I. INTRODUCTION AND METHODS.

MY preceding papers on artificial parthenogenesis[1] had proved that by an increase in the osmotic pressure of the sea-water the eggs of many, if not all, Echinoderms can be caused to develop parthenogenetically. Two new problems presented themselves for immediate consideration. The one was to raise the parthenogenetic larvæ until they were sexually differentiated, in order to decide whether or not they are of uniform sex. The second problem was to try whether artificial parthenogenesis is confined to the group of

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[1] LOEB, J.: This journal, 1899, iii, p. 135; 1900, iii, p. 434; 1900, iv, p. 178; Science, 1900, xi, p. 612.

Echinoderms or whether it is a more general phenomenon. As the means for the raising of sea-urchins were not available at Woods Holl this year, the former problem had to be postponed. The solution of the second problem, however, was possible, and yielded the result that the unfertilized eggs of Chætopterus, a marine annelid, can be caused to develop into swimming ciliated larvæ (trochophores). A short preliminary report of this result has been published in Science.[1]

In experiments on parthenogenesis the greatest precautions are necessary to exclude the possibility of a contamination of the eggs by spermatozoa. I purposely selected Chætopterus for my further experiments on account of the possibility of discriminating between and separating the females and males. If the experimenter handles females and males in the same experiment or with the same instruments, it is extremely hard to avoid an infection of the eggs by sperm. I proceeded as follows in the experiments with Chætopterus. As soon as the animals were brought into the laboratory by the collector, the tubes in which they live were opened and the worms removed. As soon as the first female was found it was put into a special dish and thoroughly washed off with sea-water, the water being renewed from six to twelve times in succession. The sea-water in the laboratory was found to be absolutely free from spermatozoa of Chætopterus. (The animals are found on the beach of an island at some distance from the laboratory.) After the female had undergone the process of washing, it was exposed to a current of sea-water over night to remove as far as possible any spermatozoa that might have been left on the surface. The next day the animal was ready to be used for an experiment. On that day and before the experiment began, the experimenter did not bring his hands in contact with any other Chætopterus or with the aquarium that contained such animals. His hands and instruments were sterilized with fresh water. The posterior part of the animal which contains the eggs was cut off and thoroughly washed for two minutes in distilled or fresh water. Had any spermatozoon been left on the surface of the animal, the distilled water would have killed it. After this the part containing the eggs was put into a vessel with sterilized sea-water, washed off once more and then put into another dish containing sterilized sea-water. In this dish the single parapodia were opened successively, the eggs sucked out from each with a pipette, and then collected in another dish

[1] LOEB, J.: *Science*, 1900, xii. p. 170.

with sterilized sea-water. After all the eggs had been collected they were divided into two lots. The one lot remained in normal (sterilized) sea-water, to serve as control material. The other lot was distributed into the various solutions whose effect I intended to test. *In no case did I see a single egg of the control material develop into a larva.* I noticed only that after from 7 to 10 hours some of these eggs may show a beginning of a segmentation which, however, soon ceases. This phenomenon seems to be quite common among many marine animals. I mentioned in a former paper that O. Hertwig had already noticed that it is a common occurrence among Arthropods, Worms, and Echinoderms.[1] If, however, no such aseptic measures against spermatozoa were taken, a number of eggs in the control material usually reached the trochophore stage. The sea-water used in these experiments was sterilized by heating it slowly to a temperature of from 60 to 80 C. In a smaller number of experiments I used sea-water which had gone through a Pasteur (Chamberland) filter which, of course, is absolutely impermeable for spermatozoa.[2] If the eggs of more than one female were used for an experiment, all the eggs were first gathered in one dish, thoroughly mixed and then divided into two lots, one to serve as control material and one to be distributed into the various solutions. Thus the control material and the material experimented upon consisted always of the eggs of the same females. It goes without saying that the same was the case in all my previous experiments on Echinoderms.

II. Artificial Parthenogenesis caused by an Increase in the Osmotic Pressure of the Sea-water.

It was natural to try first whether or not the same means that cause the parthenogenetic development in Echinoderms are also sufficient to bring about the parthenogenetic development of the eggs of Chætopterus.

First series. — When I received the first material, I at once started an experiment, although I knew that it was practically impossible to exclude contamination by spermatozoa if I attempted to isolate the eggs immediately after having handled a male. The female was

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[1] Hertwig, O.: Die Zelle und die Gewebe, 1893, i, p. 239.

[2] In almost all the experiments the sea-water used was sterilized. In a few exceptions this precaution was purposely omitted in order to find out whether or not the sea-water in the laboratory contained spermatozoa of Chætopterus. This, however, was not the case.

washed off in sterilized sea-water, but of course I was aware that this would not suffice to get rid of any spermatozoa that might be sticking to the surface of the animal. The eggs, however, were taken and distributed into the following five solutions: —

1.   6 c.c. $2\frac{1}{2}$ n KCl + 94 c.c. sea-water.
2.   8 c.c. $2\frac{1}{2}$ n KCl + 92 c.c. sea-water.
3.   10 c.c. $2\frac{1}{2}$ n KCl + 90 c.c. sea-water.
4.   12 c.c. $2\frac{1}{2}$ n KCl + 88 c.c. sea-water.
5.   Normal sea-water (control).

One part of the eggs remained 1 hour and 25 minutes, the rest 1 hour and 40 minutes in the solutions. The experiment was started in the afternoon. The next morning[1] I found numerous swimming larvæ (trochophores) in the material that had been in the first four solutions for 1 hour and 25 minutes. In the second lot they were less numerous. But even in the control material I found two swimming trochophores. It followed that the Chætopterus were either naturally parthenogenetic or the precautions against the entrance of spermatozoa had not been sufficient.

Second series. — From now on I applied the rigid antiseptic measures against spermatozoa described above in the introduction. The following solutions were used: —

1.   8 $2\frac{1}{2}$ n KCl   + 92 sea-water.
2.   10 $2\frac{1}{2}$ n KCl   + 90 sea-water.
3.   12 $2\frac{1}{2}$ n KCl   + 88 sea-water.
4.   12 $2\frac{1}{2}$ n NaCl   + 88 sea-water.
5.   20 $2\frac{1}{2}$ n $MgCl_2$ + 80 sea-water.
6.   Normal sea-water (control).

All the sea-water had been sterilized the previous day by heating it to a temperature of 80°; one part (*a*) of the eggs remained 1 hour, a second part (*b*) 1 hour and 20 minutes in these solutions.

The first four solutions yielded numerous swimming trochophores; their number was greatest in the first two solutions. Lot *a* of the $MgCl_2$ solution yielded no swimming blastulæ, but lot *b* had a few. The control eggs were completely undeveloped, with the exception that after about ten hours a few showed the beginning of a segmentation, which in no case led to the formation of more than from 4 to 6

[1] I shall in the following description of the experiments consider only whether or not swimming trochophores were formed. The morphological details will be given in Chapter V. It goes without saying that all the experiments deal with *unfertilized* eggs, unless the contrary is distinctly stated.

cells. During the next 48 hours no further development occurred, and the eggs died and disintegrated. According to this experiment the unfertilized eggs of Chætopterus are not able to develop in normal sea-water. They can, however, be caused to develop into trochophores if exposed for about an hour to sea-water whose concentration has been raised through the addition of the right quantity of KCl or NaCl.

Third series. — The next task was to ascertain how much the osmotic pressure of the sea-water must be raised in order to bring about the parthenogenetic development, and whether the increase in osmotic pressure necessary for this purpose was the same in each case. The solutions used were as follows : —

1. 10 $2\frac{1}{2}$ n KCl + 90 sea-water.
2. $12\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $87\frac{1}{2}$ sea-water.
3. 30 2 n cane sugar + 70 sea-water.
4. $12\frac{1}{2}$ $2\frac{1}{2}$ n NaCl + $87\frac{1}{2}$ sea-water.
5. Normal sea-water (control).

The osmotic pressure in solutions 2, 3, and 4 was about the same. The eggs remained 65 minutes in these solutions, and were then put back into normal sea-water. While a great number of the eggs that had been in solutions 1 and 2 developed into trochophores, very few of the eggs of solution 4 and none of solution 3 reached the trochophore stage. The control eggs remained undeveloped.

Fourth series. — The results were obviously puzzling if the increase of the osmotic pressure was the only factor that brought about the development of the unfertilized eggs of Chætopterus. But they would be intelligible if there were in addition to the effect of an increase in the osmotic pressure, a specific effect of the KCl or the K-ions. In order to decide this, the unfertilized eggs of a female were distributed into the following solutions : —

1. 5 $2\frac{1}{2}$ n KCl + 95 sea-water.
2. 10 $2\frac{1}{2}$ n KCl + 90 sea-water.
3. 15 $2\frac{1}{2}$ n KCl + 85 sea-water.
4. 5 $2\frac{1}{2}$ n NaCl + 95 sea-water.
5. 10 $2\frac{1}{2}$ n NaCl + 90 sea-water.
6. 15 $2\frac{1}{2}$ n NaCl + 85 sea-water.
7. Normal sea-water (control).

The eggs remained 1 hour in these solutions.

The next day the control eggs (7) were undeveloped. The eggs that had been in the first three solutions were teeming with trochophores. In lots 4 and 5 not a single swimming trochophore was found, al-

though many eggs had begun to develop. Their development stopped, however, in an early stage. Of the eggs that had been in solution 6 a large number had reached the trochophore stage and were swimming. These results were as clear as could be desired. In order to bring about artificial parthenogenesis through the addition of NaCl, 15 c.c. of the $2\frac{1}{2}$ n solution had to be added, while 5 c.c. of a $2\frac{1}{2}$ n KCl solution were sufficient.

Fifth series. — There was a possibility that the effect produced by NaCl was a specific Na effect, and not an effect of the increase in osmotic pressure. An experiment with cane-sugar could decide this question. My stock solution of cane-sugar was a 2 n solution, while my NaCl solution was $2\frac{1}{2}$ n. On account of the electrolytic dissociation, more than 30 c.c. of the cane-sugar solution were required to produce the same increase of osmotic pressure as by 15 c.c. of the $2\frac{1}{2}$ n NaCl solution. The following solutions were tried : —

1. 40 c.c. $2n$ cane sugar — 60 c.c. sea-water.
2. 20 c.c. $2n$ cane sugar + 80 c.c. sea-water.
3. 10 c.c. $2n$ cane sugar + 90 c.c. sea-water.
4. 10 c.c. $2\frac{1}{2}$ n KCl   + 90 c.c. sea-water.
5. Normal sea-water (control).

The eggs remained 55 minutes in these solutions. Eight hours later swimming ciliated trochophores were found in the eggs that had been in solutions 1 and 4. In 2 and 3 there were no swimming larvæ. In the control material all the eggs were still spherical and unsegmented. The next morning about 25 per cent of the eggs that had been in solution 1 swam about in the most lively manner. A few trochophores were found among the eggs that had been in solution 2. But the control eggs and the eggs that had been in solution 3 had in the best case only reached the earliest stages of segmentation. This leaves no doubt that an increase in the osmotic pressure of the sea-water is sufficient to bring about artificial parthenogenesis in the eggs of Chætopterus.

Sixth series. — In order to make this conclusion stronger, it was necessary to try the effect of an increase in the osmotic pressure of the sea-water by the addition of still other substances. The following were tried : —

1. 5 5 n CaCl$_2$ + 95 sea-water.
2. 10 5 n CaCl$_2$ + 90 sea-water.
3. 10 $2\frac{1}{2}$ n MgCl$_2$ — 90 sea-water.
4. 20 $2\frac{1}{2}$ n MgCl$_2$ · 80 sea-water.
5. 30 $2\frac{1}{2}$ n MgCl$_2$ + 70 sea-water.
6. Normal sea-water (control).

The eggs remained in these solutions 1 hour, and were then put back into normal sea-water. I neglected to look at them the same evening. The next morning I found a small number of swimming larvæ among the eggs that had been in solution 5 (30 $2\frac{1}{2}$ n MgCl^2 + 70 sea-water). The control eggs were undeveloped, the Ca$_2$ eggs had gone to pieces. The experiment demonstrated only that the increase of the osmotic pressure through MgCl$_2$ can bring about the development of the unfertilized eggs of Chætopterus.

Seventh series. — I suspected that my failure to get swimming trochophores from a mixture of sea-water and a 5 n CaCl$_2$ solution might have been due to the poisonous effect of the calcium, having noticed in my previous experiments on the artificial parthenogenesis in sea-urchins that the parthenogenetic larvæ produced by the addition of CaCl$_2$ to sea-water soon died. I therefore started a new experiment early in the morning and watched the eggs during the day. I found indeed that an increase in the osmotic pressure of the sea-water by the addition of CaCl$_2$ leads to the formation of swimming trochophores from the unfertilized eggs of Chætopterus. The solutions used were as follows : —

1. $2\frac{1}{2}$ c.c. 5 n CaCl$_2$ + $97\frac{1}{2}$ c.c. sea-water.
2. 5 c.c. 5 n CaCl$_2$ + 95 c.c. sea-water.
3. 10 c.c. 5 n CaCl$_2$ + 90 c.c. sea-water.
4. 15 c.c. 5 n CaCl$_2$ + 85 c.c. sea-water.
5. 20 c.c. $2\frac{1}{2}$ n MgCl$_2$ + 80 c.c. sea-water.
6. Normal sea-water (control).

The eggs were exposed to these solutions for 50 minutes. After 9 hours the eggs that had been in solution 3 contained living trochophores which died during the night. None of the other solutions gave rise to swimming trochophores.

Eighth series. — As there was no more doubt left that the increase in the osmotic pressure of the sea-water or the loss of a certain amount of water on the part of the egg caused the parthenogenetic development of the eggs of Chætopterus in these experiments, it now remained to ascertain how long the eggs must remain in these solutions in order to develop. I put the unfertilized eggs of a female into a mixture of 85 sea-water + 20 $2\frac{1}{2}$ n NaCl. The first lot were taken out of this mixture after 10 minutes, the second after 30, the third after 60, the fourth after 90, and the fifth after 120 minutes. The same evening (9 hours later) I found swimming trochophores among the eggs that had been taken out of the third and fourth lots. The eggs of the first lot did not show any trace

of development at that time. Of those of the second lot about one in a hundred had begun to develop. In the fifth lot the eggs had apparently undergone development, but I found no swimming larvæ. The eggs had possibly been injured by their long stay in the more concentrated sea-water.

The next morning about 20 to 40 per cent of the eggs of the third and fourth lots were swimming about in the trochophore stage. The rest did not contain any living larvæ, although some of the eggs were in the early segmentation stages.

It is therefore necessary to leave the unfertilized eggs of Chætopterus more than 30 and less than 120 minutes in a mixture of 85 sea-water and 20 $2\frac{1}{2} n$ NaCl in order to cause them to reach the trochophore stage.

Conclusions. — From these experiments we are allowed to draw the following conclusions : —

1. The unfertilized eggs of Chætopterus do not reach the trocho-phore stage if left in normal sea-water, provided the proper pre-cautions are taken against contamination by spermatozoa. Such eggs show no change during the first 7 to 9 hours, but may begin to segment after that time. In such cases the segmentation as a rule does not proceed beyond the 2- to 4-cell stages, but may in ex-ceptional cases go as far as the 12- to 16-cell stages. We may say that Chætopterus possesses a higher degree of parthenogenetic ten-dency than the Arbacia egg, which begins to segment later, after about 20 hours, and does not proceed beyond the 2- to 4-cell stage.

2. The unfertilized eggs of Chætopterus are able to develop into swimming trochophores if they are put for about one hour into one of the following solutions and then put back into normal sea-water :

      1. 15–20 $2\frac{1}{2} n$ NaCl + 85 sea-water.
      2. 40 $2 n$ cane sugar + 60 sea-water.
      3. 30 $2\frac{1}{2} n$ $MgCl_2$ + 70 sea-water.
      4. 10 $5 n$ $CaCl_2$ + 90 sea-water.

All these solutions have one element in common, namely, the about equal increase of the osmotic pressure. It seems therefore justifiable to assume that the increase in the osmotic pressure or the loss of water on the part of the egg is the cause of the partheno-genetic development of these eggs.

3. KCl or perhaps the K-ions seem to possess a specific effect upon the eggs of Chætopterus. We shall discuss this fact more fully in the next chapter.

Objections considered. — The possible objection that the eggs of Chætopterus are naturally parthenogenetic in normal sea-water or that spermatozoa had contaminated the sea-water is rendered impossible through the behavior of the control eggs and the antiseptic precautions taken. As far as I can see, there is only one objection left, which, however, although far-fetched and highly improbable, shall be considered. It might be argued that Chætopterus is hermaphroditic, but that the eggs and spermatozoa do not mature simultaneously. This prevents the fertilization of eggs in normal sea-water. But the increase in the osmotic pressure of sea-water might increase the motility or fertilizing power of the spermatozoa. The contrary is, however, true. The eggs of the same female were divided into two lots. The one was put into normal sea-water, the other was exposed for 50 minutes to a mixture of 70 sea-water + 30 $2\frac{1}{2}$ n MgCl$_2$. At about the same time the sperm of one male was distributed into two solutions of exactly the same character.

After 50 minutes the eggs that had been in normal sea-water were divided into three portions. To the first portion was added sperm from the normal sea-water; to the second was added sperm that had been for 50 minutes in a mixture of 30 c.c. $2\frac{1}{2}$ n MgCl$_2$ + 70 c.c. sea-water. To the third portion no sperm was added; it was intended to serve as control material. The result was as striking as could be desired. While the eggs to which the sperm from the normal sea-water had been added developed without exception into trochophores, not one egg developed in lot 2, to which the MgCl$_2$ sperm had been added. The control eggs remained likewise undeveloped.

A number of the unfertilized eggs that had been in the MgCl$_2$ for 50 minutes reached the trochophore stage.

This experiment proves conclusively that the MgCl$_2$ solution annihilates or certainly diminishes the fertilizing power of spermatozoa. In a previous series of experiments I had been able to show that the same is true for the eggs of sea-urchins.

In addition I convinced myself through microscopic examinations that the females used were not hermaphroditic.

III. The Specific Effect of K-ions on the Development of the Unfertilized Eggs of Chætopterus.

The preceding experiments seemed to indicate that KCl has a specific effect upon the development of the unfertilized eggs of

Chætopterus. Mead had already found [1] that if $\frac{1}{2}$ per cent KCl is added to sea-water the unfertilized eggs of Chætopterus throw out their polar bodies, while the addition of $\frac{1}{2}$ per cent NaCl to sea-water produces no such effect. It seemed of interest to find out whether the K-ions were possibly able to cause the parthenogenetic development of Chætopterus larvæ without the osmotic pressure of the sea-water being raised.

Ninth series. — The following mixtures were prepared : —

1. 10 2$\frac{1}{2}$ n KCl + 90 sea-water.
2. 5 2$\frac{1}{2}$ n KCl + 95 sea-water.
3. 2$\frac{1}{2}$ 2$\frac{1}{2}$ n KCl + 98 sea-water.
4. Normal sea-water (control).

The eggs remained in the solutions one hour. The sea-water used had been sterilized by heating it to a temperature of 80° C., as in all the previous experiments.

The next morning each of the first three lots contained a large number of free swimming larvæ, while the control material contained none.

Tenth series. — I intended to find out the minimum amount of KCl necessary to bring about artificial parthenogenesis. Moreover, I wished to know whether the addition of KCl to sea-water did not act more quickly upon the eggs than an increase in the osmotic pressure by some other substance. Seven solutions were used : —

1. $\frac{1}{2}$ c.c. 2$\frac{1}{2}$ n KCl + 99$\frac{1}{2}$ c.c. sea-water.
2. 1 c.c. 2$\frac{1}{2}$ n KCl + 99 c.c. sea-water.
3. 2 c.c. 2$\frac{1}{2}$ n KCl + 98 c.c. sea-water.
4. 10 c.c. 2$\frac{1}{2}$ n KCl + 90 c.c. sea-water.
5. 1 c.c. 2$\frac{1}{2}$ n NaCl + 99 c.c. sea-water.
6. 2 c.c. 2$\frac{1}{2}$ n NaCl + 98 c.c. sea-water.
7. Normal sea-water (control).

One lot of eggs remained in these solutions from 5 to 10 minutes, the others from 60 to 70 minutes.

The results were as follows: None of the two lots that had been in solution 1 reached the trochophore stage. None of the eggs that had been only 5 minutes in the second solution reached the trochophore stage. But the lot of eggs that had remained 1 hour in the second solution yielded a small number of swimming trochophores. The eggs that had been in the third and fourth solutions differed widely from the preceding lots. They were teeming with swimming trochophores, those that had been in these solutions 5 minutes as well as those that had been in the solutions 1 hour.

[1] MEAD : Biological lectures from the Marine Biological Laboratory of Woods Holl, Boston, 1898.

The control eggs and the eggs of lots 5 and 6 did not develop, although a number went through the first stages of segmentation.

I had observed in my former experiments that the eggs of sea-urchins can develop parthenogenetically if left permanently in sea-water whose concentration is raised but little. If the eggs of sea-urchins are put for 2 hours into a mixture of 92 sea-water + 8 $2\frac{1}{2}$ n NaCl, they will not develop into blastulæ when put back into normal sea-water: but if left for some time or permanently in such a solution, a small number of blastulæ may be formed. A number of the unfertilized Chætopterus eggs were left permanently in the solutions 1-6. The next morning the eggs that had been left in solution 2 (1 KCl + 99 sea-water) had swimming trochophores. The eggs in solution 1 did not reach the trochophore stage. In the other solutions everything was dead.

Eleventh series.—This series was practically a repetition of the preceding one, with the exception that the eggs remained from 20 to 30 minutes in the solutions, which were as follows:—

1. 1 $2\frac{1}{2}$ n KCl + 99 sea-water.
2. $1\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $98\frac{1}{2}$ sea-water.
3. 2 $2\frac{1}{2}$ n KCl + 98 sea-water.
4. 10 $2\frac{1}{2}$ n KCl + 90 sea-water.
5. Normal sea-water (control).

The eggs that had been in solution 1 had very few swimming trochophores, those that had been in solutions 2 and 3 had many, and those that had been in solution 4 still more. The control eggs were mostly undeveloped; a small number were segmented into from 2 to 16 cells.

Twelfth series.—In the experiments thus far mentioned a $2\frac{1}{2}$ n KCl solution had been added to normal sea-water. As the osmotic pressure of the sea-water is about equal to that of a $\frac{5}{8}$ n KCl solution, in all these experiments with KCl there was a rise in the osmotic pressure of the sea-water. I now wished to try whether this increase in osmotic pressure is essential for the KCl effect, or whether a mere increase in the number of K-ions without an increase in the osmotic pressure of the sea-water is able to bring about the parthenogenetic development of the eggs of Chætopterus. The solutions used were as follows:—

1. 2 $2\frac{1}{2}$ n KCl + 91 sea-water + 7 distilled water.
2. 3 $2\frac{1}{2}$ n KCl + 86 sea-water + 11 distilled water.
3. 2 $2\frac{1}{2}$ n KCl + 98 sea-water.
4. 3 $2\frac{1}{2}$ n KCl + 97 sea-water.
5. 5 $2\frac{1}{2}$ n MgCl$_2$ + 95 sea-water.
6. Normal sea-water (control).

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The eggs remained in the solutions 55 minutes. Nine hours later (the same evening) swimming trochophores were found in those that had been in the first four solutions. The eggs that had been in the other two solutions were entirely undeveloped. Some had been left *permanently* in these solutions. Some of those left in solutions 1 and 2 had reached the trochophore stage and were swimming about.

The next morning these results were confirmed. Fully one-third of all the eggs that had been 55 minutes in solutions 1-4 swam about as trochophores. Those that had been in solutions 5 and 6 had not reached the trochophore stage; only a few eggs had begun to segment.

Thirteenth series. — It was evident that the KCl brought about artificial parthenogenesis, even if the osmotic pressure of the sea-water was *not* raised. I now tried whether a pure KCl solution was able to cause artificial parthenogenesis, and whether this was possible when the osmotic pressure of such a solution was lower than that of sea-water. The solutions used were as follows: —

1. 10 $2\frac{1}{2}$ n KCl + 90 distilled water.
2. 20 $2\frac{1}{2}$ n KCl + 80 distilled water.
3. 25 $2\frac{1}{2}$ n KCl + 75 distilled water.
4. 2 $2\frac{1}{2}$ n KCl + 98 sea-water.
5. Normal sea-water (control).

The osmotic pressure of solutions 1 and 2 was smaller than that of normal sea-water. One portion was left 13, the other 50 minutes in these solutions.

The next morning a large number of swimming trochophores was found in every one of the dishes that contained eggs taken from the first four solutions. The control solutions were absolutely free from trochophores.

Fourteenth series. — The experiment was so surprising that I wished to repeat it. The following solutions were prepared: —

1. 10 $2\frac{1}{2}$ n KCl + 90 distilled water.
2. 2 $2\frac{1}{2}$ n KCl + 98 sea-water.
3. Normal sea-water (control).

The eggs were left in the solutions 50 minutes. The water was sterilized. The next morning the eggs that had been in solutions 1 and 2 contained living larvæ, while the eggs in the normal sea-water were undeveloped.

Fifteenth series. — I next wished to know whether the KCl-solution

might be still more diluted without annihilating its effect upon the unfertilized Chætopterus eggs. Four solutions were used: —

1.   5 c.c. 2½ *n* KCl + 95 c.c. distilled water.
2. 10 c.c. 2½ *n* KCl + 90 c.c. distilled water.
3. 15 c.c. 2½ *n* KCl + 85 c.c. distilled water.
4. Normal sea-water (control).

The eggs were left in these solutions 7 minutes and were then put back into sterilized sea-water. The next morning about one per cent of the eggs that had been in solution 1 were swimming about. The eggs that had been in solution 2 had practically all reached the larva stage, although not all of them were swimming. The eggs that had been in the third solution contained swimming larvæ, but fewer than the other two lots. The control eggs had remained absolutely unsegmented during the first 9–10 hours. They showed, however, a beginning of segmentation (2–3 cells) the next morning.

Sixteenth series. — There was no longer any doubt concerning the fact that KCl is able to bring about the development of the unfertilized eggs of Chætopterus. It was, moreover, apparent from experiment 10 that if only ½ c.c. 2½ *n* KCl is added to 99½ c.c. of sea-water, no trochophores are formed. It was natural to conclude from this that a certain minimal amount of K-ions must enter the egg in order to make it reach the trochophore stage. In order to decide this the following experiment was tried. The eggs of one female were put into a solution of 2 2½ *n* KCl + 98 sea-water, and put back into normal sea-water at various intervals, viz., after 1 minute, 3 minutes, 7 minutes, 9 minutes, 13 minutes, 20 minutes, 40 minutes. The results of this experiment were as definite as could be desired.

After from 9 to 10 hours the eggs of the first lot (that had been in the KCl-sea-water for 1 minute only) were absolutely unsegmented. In the second lot (3 minutes) a few eggs were segmented, but no trochophore was formed. Lot 5 (13 minutes) had trochophores which did not yet move, and in 6 (20 minutes) and 7 (40 minutes) trochophores were found that were just beginning to move. The control eggs were absolutely unsegmented.

The next morning I found *no* trochophores in the first lot (1 minute), but many eggs in a 2- to 8-cell stage. In lot 2 (3 minutes) about 1 per cent of the eggs were swimming about as trochophores. In lot 4 about 10 per cent of all the eggs were swimming about as trochophores; in lot 5 (13 minutes) it was about the same.

In lot 6, whose eggs had been for 20 minutes in the KCl-sea-water, about 50 per cent swam about in the trochophore stage. Lot 7 seemed to contain not quite so many trochophores.

It is therefore necessary that the unfertilized eggs remain more than 1 minute in a mixture of 2 $2\frac{1}{2}$ n KCl + 98 sea-water in order to develop; three minutes (or possibly a little less) is sufficient. This indicates clearly that a certain quantity of K or KCl must enter the egg in order to bring about the development. This quantity is very small. It seems to vary, however, for the individual eggs, inasmuch as the number of eggs that developed was greater the longer the eggs remained in the KCl solution. If they remain too long in such a solution, the KCl acts like a poison. From 20 to 60 minutes seems to be the optimal time.

Seventeenth series. — I wished to determine once more what was the smallest amount of KCl that must be added to sea-water in order to bring about artificial parthenogenesis of the Chætopterus eggs. The following solutions were used: —

1. $\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $99\frac{1}{2}$ sea-water.
2. 1 $2\frac{1}{2}$ n KCl + 99 sea-water.
3. $1\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $98\frac{1}{2}$ sea-water.
4. Normal sea-water (control).

The eggs were left in these solutions over night. The next morning, after they had been in these solutions 24 hours, the first solution contained many eggs in the beginning stages of segmentation, but not one swimming larva could be discovered. The second and third solutions contained a large number of swimming larvæ; in the second they were more numerous than in the third. In the control material only a few eggs began to segment; no swimming larvæ were to be found. This confirms our former observation that an addition of $\frac{1}{2}$ $2\frac{1}{2}$ n KCl to $99\frac{1}{2}$ sea-water is insufficient to produce parthenogenesis, while the addition of 1 KCl is sufficient.

Eighteenth series. — There is something paradoxical in the fact that the addition of 2 $2\frac{1}{2}$ n KCl to 98 sea-water can produce parthenogenesis in 3 minutes, while the addition of $\frac{1}{2}$ $2\frac{1}{2}$ n KCl to $99\frac{1}{2}$ sea-water cannot accomplish the same result in 24 hours. Before I accepted this as a fact I wished to see it confirmed once more. The same solutions were applied as before: —

1. $\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $99\frac{1}{2}$ sea-water.
2. 1 $2\frac{1}{2}$ n KCl + 99 sea-water.
3. $1\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $98\frac{1}{2}$ sea-water.
4. Normal sea-water (control).

Part of the eggs were put back into normal (sterilized) sea-water after 30 minutes, while the others remained in the solution during the next 24 hours. As far as the latter are concerned the results were exactly like those described in the preceding experiment. The eggs that had remained in solution 1 over night had not developed beyond the early cleavage stages. No egg had reached the trochophore stage. In the second solution a large number of swimming larvæ were found, and in the third solution they were numerous. About 75 per cent of all the eggs were in the trochophore stage, and many of these were swimming about.

The eggs that had remained in these solutions only 30 minutes showed the following condition the next morning. Those that had been for 30 minutes in the first solution had no trochophores; only a few had begun to segment. The eggs that had been taken out of solution 2 after 30 minutes had formed many larvæ, but fewer than the eggs that had remained in the solution. Those that had been taken out of solution 3 after 30 minutes had formed many swimming larvæ. The control eggs were undeveloped, save a few that had begun to segment.

While a stay of 30 minutes in a mixture of 1 c.c. $2\frac{1}{2}$ KCl + 99 c.c. sea-water suffices to cause the eggs to develop parthenogenetically, a stay of 30 hours in a solution of $\frac{1}{2}$ $2\frac{1}{2}$ n KCl + $99\frac{1}{2}$ sea-water remains without any effect. This may mean that a minimal quantity of K or KCl must enter the eggs in a certain minimal time or rather suddenly. It may, however, find a different explanation.

Nineteenth series. — Is the fertilizing power of the KCl due to the K-ions or to the KCl molecules? The unfertilized eggs of one female were distributed into the following solutions: —

1. 1 $2\frac{1}{2}$ n KBr   + 99 sea-water.
2. 2 $2\frac{1}{2}$ n KBr   + 98 sea-water.
3. 1 $2\frac{1}{2}$ n KNo$_3$ + 99 sea-water.
4. 2 $2\frac{1}{2}$ n KNo$_3$ + 98 sea-water.
5. 3 $\frac{1}{2}$ 2 n K$_2$SO$_4$ + 97 sea-water.
6. Normal sea-water (control).

The eggs remained in these solutions 30 minutes, and were then put back into normal sea-water. The eggs that had been in solutions 2, 4, and 5 formed a large number of swimming larvæ, the others remained undeveloped. This experiment proves that the K-ions and not the KCl molecules produce the parthenogenetic development of the eggs of Chætopterus.

Conclusions. — These experiments confirm the conclusion drawn above that the unfertilized eggs of Chætopterus cannot develop into a trochophore if left in normal sea-water. A small number of K-ions, however, is able to cause them to develop parthenogenetically. If the eggs are put for 3 minutes into a mixture of 2 c.c. $2\frac{1}{2}$ n KCl + 98 c.c. sea-water, they are able to develop parthenogenetically. If the sea-water contains fewer K-ions, *e.g.*, if we add 1 c.c. $2\frac{1}{2}$ n KCl to 99 c.c. sea-water, the eggs must remain longer in the solution. Finally, if we add only $\frac{1}{2}$ c.c. $2\frac{1}{2}$ n KCl to $99\frac{1}{2}$ c.c. sea-water, the eggs are not able to develop parthenogenetically, no matter how long they are left in such a solution. They can be caused to reach the trochophore stage by a pure KCl solution of considerably lower osmotic pressure than that of sea-water. If the sea-water contained only a slightly greater proportion of K, we should find that Chætopterus was "normally" parthenogenetic.

IV. ARTIFICIAL PARTHENOGENESIS PRODUCED BY A SLIGHT ADDITION OF HCL TO SEA-WATER.

In my experiments on Echinoderms, I had found that the addition of a small quantity of acid or alkali causes the unfertilized eggs of sea-urchins to segment much more quickly than is the case in normal sea-water. I intended to try the effects of the same agencies on the eggs of Chætopterus. The sea-water is slightly alkaline, *i.e.*, has a small quantity of free hydroxyl-ions in solution. If we add more alkali, the number of the hydroxyl-ions is but slightly increased, inasmuch as a precipitate of $Mg(HO)_2$ is formed. With acids it is different. If we add a certain small amount, the sea-water becomes neutral; and if we add more, it becomes acid according to the amount and degree of dissociation of the acid used. All the sea-water in these experiments was sterilized.

Twentieth series. — The following solutions were used : —

1. 100 c.c. sea-water + 2 c.c. $\frac{1}{10}$ n NaHO.
2. 100 c.c. sea-water + 2 c.c. $\frac{1}{10}$ n KHO.
3. 96 c.c. sea-water + 4 c.c. $\frac{1}{4}$ n Na_2CO_3.
4. 100 c.c. sea-water + 2 c.c. $\frac{1}{10}$ n HCl.
5. 100 c.c. sea-water + 3 c.c. $\frac{1}{10}$ n HCl.
6. Normal sea-water (control).

The eggs of one female were distributed in these solutions. One portion of eggs was taken out of these solutions and put back into normal sea-water; the others remained permanently in these

solutions. Twenty-four hours later the results appeared to be as follows. Of the eggs that had remained in the solutions for 24 hours, those in solutions 2 and 4 had well-developed trochophores that swam about. In solution 1 several eggs seemed to have developed, but I was unable to find one swimming or with cilia. Those in the other solutions were undeveloped. The eggs that had been in solutions 2 and 4 for only 5–10 minutes had a few trochophores, the others were undeveloped.

It is evident that KHO is more effective than NaHO, and it is natural that the effect of the K-ions should have been added to the effect of the HO-ions. But the fact is very striking that the addition of a small amount of HCl to the sea-water caused the parthenogenetic development of the Chætopterus eggs.

Twenty-first series. — The unfertilized eggs of a Chætopterus were distributed in the following solutions : —

> 1. 100 sea-water + 1 $\frac{1}{10}$ n HCl.
> 2. 100 sea-water + 2 $\frac{1}{10}$ n HCl.
> 3. 100 sea-water + 3 $\frac{1}{10}$ n HCl.
> 4. Normal sea-water (control).

One portion of eggs remained in the solutions 5 minutes, the others permanently. The eggs that were taken from the solutions after 5 minutes were undeveloped, with the exception of a few that had been in solution 2 and which reached the trochophore stage. The eggs that remained permanently in solutions 1 and 2 formed a large number of swimming larvæ. The eggs in solutions 3 and 4 were undeveloped and dead.

Twenty-second series. It was evident that the addition of 2 c.c. $\frac{1}{10}$ n HCl to 100 c.c. sea-water was able to cause the development of the unfertilized eggs of Chætopterus, especially if the eggs remained permanently in this solution. I intended to see how long the eggs must remain in such a solution in order to reach the trochophore stage. Eggs were put into such a solution and taken out in intervals of 10, 30, 60, 90, and 120 minutes, respectively. One portion remained there permanently. A large number of swimming larvæ developed only in the latter portion; in the former there were none. The control eggs remained absolutely undeveloped.

Although these experiments are not yet finished, they seem to indicate that in a solution of 100 c.c. sea-water + 2 c.c. $\frac{1}{10}$ n HCl the unfertilized eggs of Chætopterus can reach the trochophore stage.

V. Morphological Observations on the Development of the Unfertilized Eggs of Chætopterus.

I have thus far confined myself to the statement that certain solutions are capable of causing the unfertilized eggs of Chætopterus to reach the trochophore stage and swim about. Nothing has been said as yet concerning the mode of development of these parthenogenetic eggs. I have watched their development very carefully and have made a number of camera drawings. This part of the work is essential for experiments on parthenogenesis. If one wishes to be absolutely certain in regard to the parthenogenetic character of the development, a close continuous observation and study of the eggs *during the first 7–9 hours is necessary.* During this time the parthenogenetic eggs throw out their polar bodies, segment, and become trochophores, while the control eggs or the eggs treated with ineffective solutions remain quite spherical and unchanged.

The egg of Chætopterus is very dark and opaque, and it is for this reason much more difficult to determine the number of cleavage cells in it than in the egg of most Echinoderms. The *fertilized* egg of Chætopterus develops very quickly. At a favorable temperature the cilia develop 5 hours after fertilization, and the larvæ begin to swim. The development of the unfertilized eggs differs in most cases from that of the fertilized eggs. It is a little slower, and the nature of the segmentation and the distinctness of the single cleavage spheres vary considerably with the nature of the ions that are added to the sea-water, or the agency employed to bring about artificial parthenogenesis. If K-salts are used, one does not, as a rule, notice much more of the beginning development, except that the eggs become irregular in their outline and amœboid. In the experiments with Ca-salts and acids, the cleavage spheres were much more distinct and regular. Fig. 1 gives a good average picture of the amœboid character of the K-eggs. In the experiment in which these eggs were drawn the unfertilized eggs of a Chætopterus were put into a mixture of 98 sea-water $+ 2\, 2\frac{1}{2}\, n$ KCl at 9.43. They remained in this solution 40 minutes, and were then put back into normal sea-water. Three hours later, at 1.40, the drawing (Fig. 1) was made. It is impossible to recognize any distinct cleavage spheres in these eggs. All that can be said is that they have lost their spherical outline and are amœboid. I have never seen anything like this in fertilized eggs, or in the unfertilized control eggs that are

left in normal sea-water. If the latter segment at all, they do not begin to do so until after 7–9 hours or later, and they form more distinct cleavage cells.

The appearance of the eggs and the form of segmentation are thus distinctly a function of the constitution of the sea-water. Inasmuch as the K-eggs give rise to trochophores which may look as normal as those developing from a fertilized egg, it is evident that the appearance of the cleavage cells is of very little importance in the formation of the embryo.

The difference between the development of unfertilized K-eggs and fertilized eggs can be seen from Fig. 2. In the experiments in which these drawings were made the eggs of one female were divided into two lots. The one was fertilized at 11.45 by the addition of sperm; the other was put at the same time for 55 minutes into a mixture of 98 sea-water + 2 $2\frac{1}{2}$ n KCl. In about 15–20 minutes after the eggs were put into this mixture they threw out their polar bodies; sometimes one, sometimes two were visible. This harmonizes with Mead's observations. In the unfertilized control eggs that had remained in normal sea-water nothing of this kind was noted.

Fifty-five minutes after the eggs had been put into the KCl mixture they were put back into normal (sterilized) sea-water. In from 10 to 30 minutes they began to lose their spherical shape, and in some eggs little processes or knobs appeared and remained or were withdrawn. The eggs resembled amœbæ in their behavior. In Fig. 2, on the left side the development of the fertilized lot is represented; on the right side the development of the unfertilized eggs. At about the same time a drawing of the fertilized and the unfertilized K-eggs was made. At 12.45 some of the fertilized eggs were found in the 4–8-cell stage. The unfertilized

FIGURE 1. — Camera drawings of typical forms of unfertilized Chætopterus eggs several hours after treatment with KCl. The eggs were put into a mixture of 98 c.c. sea-water + 2 c.c. $2\frac{1}{2}$ n KCl solution at 9.43. They remained in this solution 40 minutes, and were then put back into normal sea-water. They were drawn at 1.40. From 20 to 50 per cent of the eggs thus treated reached the trochophore stage.

Fertilized.   Unfertilized.

12.45   12.55

1.20   1.30

2.10   2.

3.40   3.15

4.30

7.40
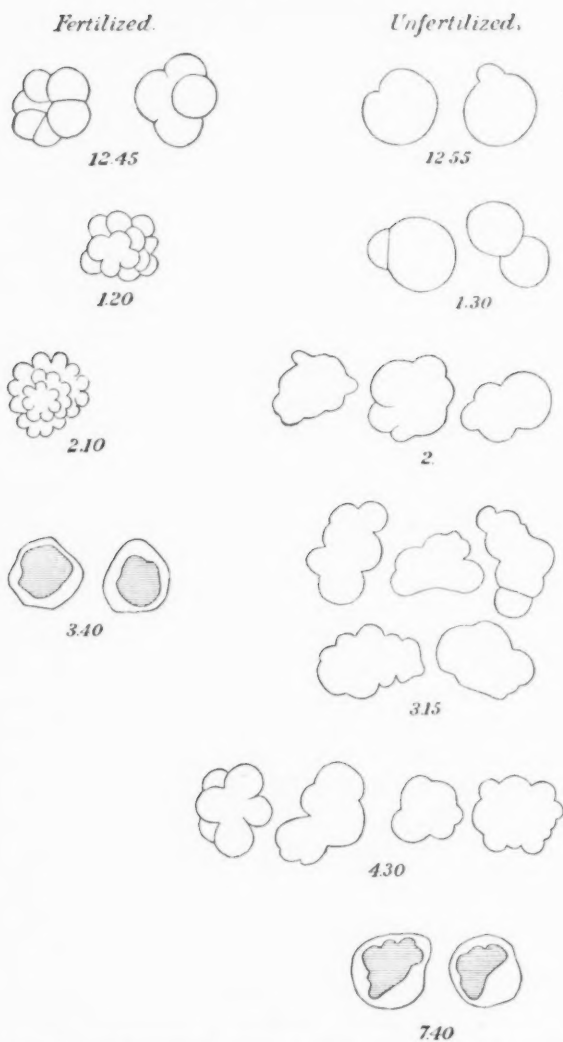
FIGURE 2. — Differences between the development of eggs fertilized by spermatozoa and unfertilized eggs treated with KCl. The eggs came from the same female. One lot was fertilized at 11.45. The other lot had been put (a few minutes earlier) for 55 minutes into a mixture of 98 sea-water + 2 $2\frac{1}{2}$ n KCl solution. On the left side the successive stages of the fertilized eggs are drawn; the right side contains the camera drawings of the unfertilized (or chemically fertilized) eggs at the same time. In each case the most advanced or most differentiated eggs were drawn. (See text.)

eggs were only amœboid at that time. Some of them (see Fig. 2, 12.55) showed an incision, as if they were about to divide. At 1.20 some of the fertilized eggs had reached the 16-cell stage, and at 1.30 only a few eggs were found among the K-eggs that seemed to be segmented. At 2.10 the fertilized eggs were in an advanced stage of cell division, while the K-eggs were not distinctly segmented. At 3.40 the fertilized eggs had reached the trochophore stage, with a clear edge and a dark centre. At that time the most differentiated eggs of the parthenogenetic lot were in the condition that is represented at 3.15 in Fig. 2. At 4.30 we find these eggs still in the same condition, and not until 7.40 did the parthenogenetic eggs reach the beginning of the trochophore stage, — clear edge and dark centre (Fig. 2). The fertilized eggs had formed their cilia, and at about 5 o'clock were swimming around, while the K-eggs did not begin to swim until 8 or 9 o'clock. The unfertilized control eggs which had remained in normal sea-water during this time were at 8 o'clock still absolutely spherical, and had given no sign of development or change.

Although the drawings in Fig. 2 give an idea of the development of the parthenogenetic eggs, this idea has to be supplemented by the statement that not all the eggs behaved like those drawn. The majority of parthenogenetic eggs never showed any higher degree of differentiation during their development than those drawn in Fig. 1; many eggs even remained spherical. The number of trochophores was always considerably larger than the number of eggs that became amœboid. The majority of parthenogenetic trochophores are perfectly spherical. I have often wondered whether it was possible for the unfertilized K-eggs to reach the trochophore stage without any visible external signs of cleavage. I shall have to postpone a definite answer to this question until next year.

Another point worth mentioning is the fact that phenomena of cleavage seem to be reversible in this form, inasmuch as an egg divides into two spheres which very soon fuse again. Such changes, which occur very suddenly, may be occasionally observed in unfertilized Chætopterus eggs. Fig. 3 shows the successive stages which were observed in one egg within 4 minutes. I had watched these lively changes for several minutes before I decided to draw them. The egg had been for an hour in a mixture of 95 sea-water + 5 2½ n NaCl, and had been back in sea-water for 8 hours. When I began to draw the egg, it had the appearance of being in the

Jacques Loeb.

2-cell stage (Fig. 3, 8.04). Ten seconds later it changed suddenly into a 3-cell stage, the upper sphere breaking into two cells (8.04½). A few seconds after this the lower sphere began to flow into the right upper sphere (8.05), and at 8.05¼ it had disappeared completely. The egg was again in the 2-cell stage (8.05¼). Then the two spheres fused, and a small sphere or droplet appeared above (8.05½). This disappeared almost immediately, and a new little droplet broke loose at the right lower side of the egg (8.06). It disappeared in a few seconds, and the egg once more divided, but with
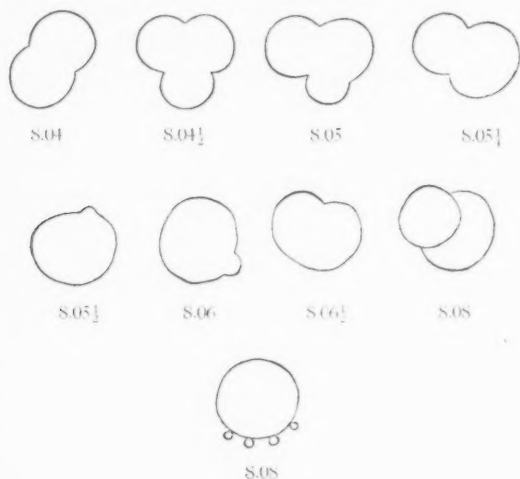
FIGURE 3. — A series of rapid successive changes in an unfertilized Chætopterus egg. Within 4 minutes the eggs appeared in the 2-cell, 3-cell, 2-cell, 1-cell, 2-cell, and 1-cell stage again. The plane of cleavage was different in each case.

an altogether different position of the cleavage plane (8.06½, 8.07½). In a few seconds the two spheres fused into one cell, and a number of small droplets appeared below (8.08). Of course it is impossible to tell whether or not these single spheres or droplets contained nuclei. These phenomena are of importance for the mechanics of development, inasmuch as they show that the bulk of the egg is liquid, and that in the case of Chætopterus its viscosity is very small and less than in the case of the sea-urchin's egg. It is hard to understand what kind of structure could be preformed in a liquid mass of such low degree of viscosity beyond the differentiation into nuclear and protoplasmic material and possibly centrosomes.

The appearance of the trochophores originating from unfertilized eggs is exactly like that of those arising from fertilized eggs, if one compares equal stages of development. Fig. 2 gives no good idea of the trochophore, inasmuch as the latter is at first spherical. Fig. 4 shows two parthenogenetic trochophores, drawn by the camera with the exception of the cilia, which are more or less diagrammatic. The eggs from which these trochophores originated had been treated with KCl. It is hardly necessary to mention that the appearance of the trochophores developing from parthenogenetic eggs depends greatly upon the treatment the egg had received. I mentioned this point in connection with the artificial parthenogenesis of sea-urchins.[1]

A point which must be discussed is the duration of life of the parthenogenetic trochophores. All the Chætopterus larvæ, those that developed from fertilized eggs as well as those that developed from unfertilized eggs, died after two days. As the fertilized eggs developed faster than the unfertilized eggs, the trochophores that had developed from the former eggs were in a more advanced stage at the time of death

FIG. 4.—Camera drawing of typical trochophores produced from unfertilized eggs by KCl treatment.

than the parthenogenetic trochophores. But to judge from the energy of their motion, the vitality of the parthenogenetic trochophores equalled that of the trochophores emanating from fertilized eggs. The cause of death was apparently the development of microorganisms in the poorly aerated culture dishes. The parthenogenetic larvæ of Arbacia lived, under similarly unfavorable conditions, as long as ten days.

VI. On the Effect of Various Ions on the Artificial Production of Parthenogenetic Giant and Dwarf Embryos in Arbacia and Chætopterus.

In a former paper on the artificial parthenogenesis of sea-urchins I have mentioned the fact that as a rule more than one embryo originates from one egg.[2] It was not unusual to see 3, 4, or even 6 blastulæ arise from one egg. Of course each of these embryos was smaller than the normal embryo of Arbacia in which the whole mass is utilized for one embryo. In my first experiments I had

[1] LOEB: This journal, 1900, iii, pp. 460, 461
[2] LOEB: This journal, 1900, iii, p. 434.

caused the parthenogenetic development of the eggs of Arbacia by raising the osmotic pressure of the sea-water through the addition of $MgCl_2$. I have since found that it depends upon the nature of the substance which is added to the sea-water whether the parthenogenetic larvæ are dwarfs or of normal size. If the unfertilized eggs of Arbacia are put into sea-water whose osmotic pressure has been raised by the addition of KCl (*e.g.*, 88 sea-water $+$ $12.2\frac{1}{2}$ n KCl), and if after 2 hours they are put back into normal sea-water, they will develop into swimming larvæ. In this case, as a rule, only one embryo develops from an egg, and dwarf larvæ are an exception. If, however, instead of KCl the corresponding quantity of NaCl or $MgCl_2$ is added to the sea-water, as a rule more than one embryo originates from one egg, and larvæ of normal size are rare. I have not made many experiments with $CaCl_2$, but it seems to act more like KCl than like NaCl. In the experiments in which the osmotic pressure of the sea-water was raised by cane sugar, dwarf blastulæ were also observed.

I have already mentioned in an earlier paper that the lack of a membrane favors the origin of more than one embryo from the unfertilized egg. The fertilized egg has a membrane which keeps the cleavage cells together. But if the membrane be destroyed, the egg may give rise to more than one embryo. In a small number of unfertilized eggs the treatment with KCl gives rise to a very thin film, which may act as a membrane and prevent the cleavage cells from becoming separated. But such a fine film is lacking in the majority of eggs treated with KCl (or $CaCl_2$) in the right proportions to produce parthenogenesis. And yet we do not notice the falling apart of cleavage cells which in the case of the NaCl eggs or $MgCl_2$ eggs leads to the formation of more than one embryo from an unfertilized sea-urchin's eggs. The observation of the process of cleavage shows that the treatment of the eggs with KCl increases their power of adhesion. The various cleavage cells of a K-egg stick together, while after a treatment with NaCl the cleavage cells adhere much less to one another and fall apart. The same tendency is produced by the addition of $MgCl_2$ to sea-water. It is quite possible that the relative amount of the various ions influences the degree of agglutination in the cleavage cells. Herbst has observed that in sea-water without Ca the cleavage cells of fertilized eggs show a tendency to fall apart.[1]

[1] HERBST, C.: Archiv für Entwickelungsmechanik, 1900, ix, p. 424.

It was to be expected that if KCl makes the cells of the same egg stick together, it might also cause several eggs to agglutinate. We know, from the experiments of Driesch[1] and Morgan[2] on the eggs of sea-urchins and of Zur Strassen[3] on the eggs of Ascaris, that if two eggs stick together they may give rise to a single embryo of
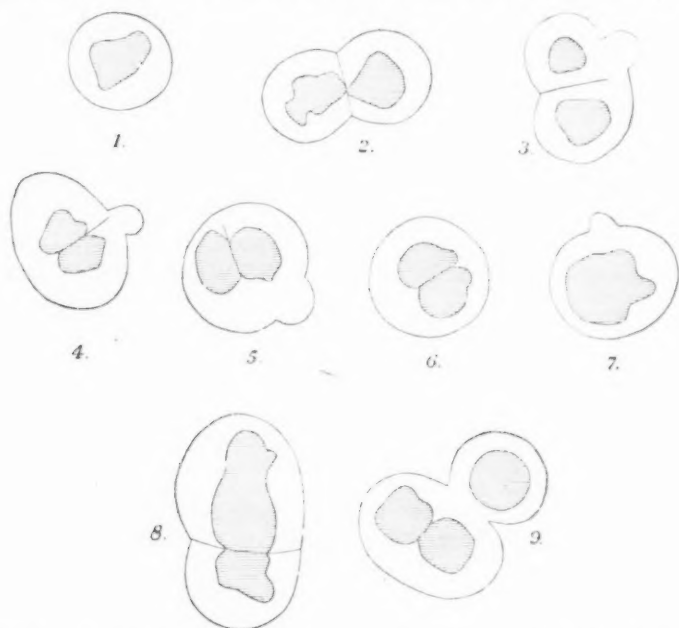
FIGURE 5. — Agglutination of Chætopterus eggs after treatment with KCl, and origin of giant embryos. The drawings were made after the eggs had reached the trochophore stage. 1. Single trochophore. 2. Two trochophores grown together but with independent organization. 3, 4, 5. The dark centres are independent, but the clear margins are partially fused. 6. The centres still independent, but the margin completely one. 7. Fusion of two eggs complete, giant embryo. 8 and 9. Fusion of three eggs.

larger dimensions. I have never observed giant embryos in the parthenogenetic eggs of sea-urchins. But I have seen them in almost every experiment in which the Chætopterus eggs had been treated with potassium. In such cases often two or more eggs would stick together, and the result was either two or more trochophores grown

[1] DRIESCH: Archiv für Entwickelungsmechanik, 1900, x, p. 411.
[2] MORGAN: Archiv für Entwickelungsmechanik, 1895, ii, p. 65.
[3] ZUR STRASSEN: Archiv für Entwickelungsmechanik, 1898, vii, p. 642.

together or a single giant embryo of twice or three times the mass
of a normal trochophore. Of course there were all kinds of transi-
tions between the two extremes. The formation of one giant embryo
through the fusion of two or more eggs is the more remarkable as
the Chætopterus eggs possess a membrane even in the unfertilized
condition. This membrane is evidently liquefied at the point of con-
tact of two eggs. The agglutination caused by K is not only notice-
able in unfertilized but also in fertilized eggs of Chætopterus. Fig. 5
shows a number of trochophores which originated from agglutinating
fertilized eggs of Chætopterus. All these and many other specimens
of this kind were found in a few drops of the culture taken out with a
pipette. I have tried to make camera drawings of the various types
that occurred. The embryos were 8 hours old, and began to move.
1 (Fig. 5) is a trochophore developed from one egg; 2 shows
two trochophores which are grown together but are otherwise inde-
pendent. In 3 we notice the beginning of a common organization,
inasmuch as the clear peripheral areas (on the right side) are fused
together. In 4 and 5 and in 6 the clear areas are almost completely
fused together, and only the dark centres remain separated. In 7
both eggs are fused completely and form one giant embryo with one
set of organs. Cases like this are very frequent in the material
treated with KCl. 8 and 9 are examples of the fusion of more than
two eggs. I have seen four eggs form one giant embryo with one
common dark centre and one common clear area. Such monsters
swam, but usually died sooner than the single embryos.

The fact that the fusion of two eggs into one giant embryo occurs
so much more readily in Chætopterus than in Arbacia may be due to
the difference in the viscosity of the two eggs.

The formation of one giant embryo from two eggs in Chætopterus
is so very interesting for the reason that the Chætopterus egg pos-
sesses a characteristic cell-lineage. We must conclude from this that
the cell-lineage is either a secondary element in the formation of the
embryo or that the earlier processes of differentiation in the Chætop-
terus egg are partly or wholly reversible (see chapter X).

I have made very few experiments with $CaCl_2$, but in these giant
embryos were formed. Eggs that had been in a solution of 90 sea-
water $+$ 10 $\frac{5}{8}$ n $CaCl_2$ for one hour, gave rise to a number of giant
embryos. A sure way to produce giant embryos in Chætopterus
is to put the unfertilized eggs for about one hour into a mixture of 97
sea-water $+ 3$ $2\frac{1}{2}$ n KCl.

I have occasionally, but very rarely, found that the fertilized eggs of Chætopterus show agglutination in normal sea-water. The same phenomenon seems to occur in the eggs of Ascaris, according to Zur Strassen.[1]

Dwarf embryos are rarely found in Chætopterus. I have found them in the experiments with HCl. Perhaps the existence of a membrane prevents the unfertilized eggs of Chætopterus from forming dwarf embryos as easily as the unfertilized eggs of the sea-urchin.

VII. On Differences between the Artificial Parthenogenesis of Echinoderms and Chætopterus and the Possibility of a Hybridization between the Two.

It is impossible to hybridize Arbacia and Chætopterus in normal sea-water. I have tried a number of experiments with negative results, as was to be expected. The negative result might be due to the impossibility of the spermatozoon of the one species entering the egg of the second species, or to the fact that the spermatozoon of Chætopterus brings about the development of the Chætopterus egg by substances which are ineffective in the Arbacia egg and *vice versa*, or the spermatozoon of the one species is poisonous for the egg of the other species, or *vice versa*.[2] The second possibility is of interest to us on account of the fact that we can bring about the parthenogenetic development of the Chætopterus eggs by means which have no effect upon the Arbacia egg.

When we intend to produce artificial parthenogenesis in the eggs of Echinoderms, it is only necessary to put them for from $1\frac{1}{2}$ to 2 hours in sea-water, the osmotic pressure of which has been raised about $37\frac{1}{2}$ to 75 per cent; that is, into sea-water to which has been added $12\frac{1}{2}$ to 25 per cent of its volume of a $2\frac{1}{2} n$ NaCl solution or of a solution isosmotic with the latter. We have not yet determined the osmotic pressure of the sea-water at Woods Holl, and on indirect data assume that it is about isosmotic with a $\frac{5}{8} n$ NaCl solution. The optimal increase of osmotic pressure varies for different species and even for different females of the same species. It may be that the temperature of the water and the degree of maturity of the eggs play a rôle. In making experiments of this kind, it is neces-

[1] Zur Strassen : *Loc. cit.*

[2] Certain constituents of the blood (globulines, enzymes?) frequently destroy the blood corpuscles of other species that are not closely related.

sary to use always a series of solutions of different osmotic pressure
and to take the eggs out at various intervals, from $\frac{1}{2}$ to 2 hours or
more, until the optimum concentration and time have been ascer-
tained.

An increase in the osmotic pressure of the sea-water is also able
to cause artificial parthenogenesis in Chætopterus. The chief differ-
ence between the Chætopterus and Arbacia eggs is that at the same
temperature the Chætopterus eggs do not need to stay so long in
the more concentrated solution as the eggs of Arbacia.

Although in this regard the difference between Chætopterus and
Arbacia is slight, a very striking difference exists in regard to the
specific effects of K-ions upon the development. While a pure KCl
solution of lower osmotic pressure than sea-water, or sea-water with a
slight increase of K, *e.g.*, a mixture of 98 sea-water + 2 $2\frac{1}{2}$ *n* KCl,
causes the parthenogenetic development of the eggs of Chætopterus
that have been exposed to such a solution only a few minutes, such
solutions are without any effect upon the unfertilized eggs of sea-
urchins (Arbacia). I left the unfertilized eggs of Arbacia repeatedly
in a mixture of 98 sea-water + 2 $2\frac{1}{2}$ *n* KCl or 97 sea-water + 3 $2\frac{1}{2}$ *n*
KCl for from 3 minutes to 24 hours without any development follow-
ing, with the exception of a few eggs that reached the 2-cell stage after
about 20 hours. But this happens just as well in normal sea-water.

As far as the Arbacia eggs are concerned, I can only state that if
we increase the osmotic pressure of the sea-water by adding KCl,
a slightly smaller increase in the osmotic pressure is required to
bring about the parthenogenetic development than if we add NaCl.
I found regularly that while 90 sea-water + 10 $2\frac{1}{2}$ *n* KCl sufficed to
cause a great many eggs to reach the blastula stage, a mixture of 90
sea-water + 10 $2\frac{1}{2}$ *n* NaCl was practically ineffective. I had to take
$87\frac{1}{2}$ sea-water + $12\frac{1}{2}$ $2\frac{1}{2}$ *n* NaCl. It is, however, possible that this
difference is only apparent. As the sea-water consists chiefly of
NaCl, the addition of 10 c.c. of a $2\frac{1}{2}$ *n* NaCl to 90 sea-water will in-
crease the osmotic pressure of the sea-water less than the addition
of 10 c.c. of a $2\frac{1}{2}$ *n* KCl solution, as the degree of dissociation is
less if the concentration is higher. Further experiments with pure
NaCl- and KCl-solutions will have to decide whether the difference
in the degree of dissociation is responsible for the result. A second
typical difference between the Arbacia egg and the Chætopterus egg
consists in the fact that the latter can be caused to develop by a
small addition of HCl to sea-water. Any other inorganic acid would

probably act in the same way, as the addition of a small amount of Cl-ions has no such effect. This small addition of acid diminishes or neutralizes the alkalinity of the sea-water, but I have failed to test whether the latter is rendered acid.

The same treatment does not cause the Arbacia eggs to develop beyond the 2-cell or 4-cell stage, even if they are left in the solution for 24 hours. I have made a number of new experiments this summer, but I have only been able to confirm the experiments mentioned in a former paper.[1]

I have pointed out that the experiments on artificial parthenogenesis force us to assume that the influence of the spermatozoon upon the development and the transmission of the qualities of the male depend upon different constituents of the spermatozoon. On the basis of this assumption the possibility of a successful hybridization between animals as far apart as Worms and Echinoderms might be considered. If we could cause the egg of Chætopterus to develop by treating it with KCl and at the same time force the spermatozoon of an Arbacia (or a similarly distant animal) to enter into the egg, we might carry Echinoderm qualities into an Annelid egg.[2] But in all my attempts at thus crossing the female Chætopterus with the male Arbacia perfect trochophores without Echinoderm characteristics resulted. Although the problem may not be capable of solution in these two forms, I think that the experiments on artificial parthenogenesis will ultimately make hybridizations possible which otherwise would be impossible. I intend to continue these experiments.

VIII. Preliminary Experiments on Phascolosoma, Fundulus, Gonionemus, and Podarke.

I will report briefly on experiments which I began but was not able to finish, partly from lack of material and partly from lack of time. My experiments on Phascolosoma were carried further than the rest. I began with putting the unfertilized eggs of this form in mixtures of 90 sea-water + 10 $2\frac{1}{2}$ n KCl and leaving them in this solution from 30 to 150 minutes. I never saw an egg reach the 2-cell stage. Then stronger solutions were tried, and now some of the eggs began to segment. When the eggs were put into a mixture of

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[1] Loeb : This journal, 1900, iii. p. 434.

[2] Provided the spermatozoon of the Echinoderm contains no poison for the Annelid egg.

about 30 2½ *n* KCl + 70 sea-water for about 30 minutes, they reached
a 30 to 60-cell stage. The appearance of the eggs was so good that
possibly in a continuation of these experiments parthenogenetic
larvæ will be produced. In these experiments I received valuable
advice from Dr. Gerould of Dartmouth College, who is thoroughly
familiar with the biology and embryology of this form.

In Fundulus, a teleost fish, I succeeded in causing the unfertilized
eggs to reach the 2-cell stage, but lack of material prevented my
carrying the experiments further.

In my experiments on Gonionemus, a medusa, I was assisted by
Dr. Murbach, who was kind enough to select the females for me. Dr.
Murbach had observed that by putting these animals into the dark
they can at any time be caused to lay eggs.

My attempts (four experiments) to cause artificial parthenogenesis
in these eggs have failed. All I was able to accomplish was to force
the eggs to become amœboid and creep about, but no segmentation
occurred.

In Podarke, an annelid, I succeeded in producing the first
segmentation in unfertilized eggs. I interrupted these experi-
ments to go on with experiments on Chætopterus which were much
more promising.

IX. Natural and Artificial Parthenogenesis.

In a definite although very small number of animals each egg
possesses the quality to develop parthenogenetically. Instances of
this are to be found in the bees, social wasps, Bombyx, Psyche,
Daphnia, plant lice, and others. In all these animals the egg can be
fertilized also by a spermatozoon. How does it happen that in
these forms, although fertilization may occur, the egg is, under
certain conditions at least, able to develop parthenogenetically?
Our experiments show that if the constitution of the sea-water were
only slightly different, that is, if it contained a little more K,
Chætopterus would have to be added to the list of normally
parthenogenetic animals. What I stated in my preliminary report is
certainly true for Chætopterus, namely, that it is the constitution
of the sea-water which prevents many or certain forms from being
" naturally " parthenogenetic. By reversing this statement we may
say that in the naturally parthenogenetic animals it may be due to the
constitution of the blood (or the sea-water?) that the egg can
develop without fertilization.

The bridge between the phenomena of natural and artificial parthenogenesis is formed by those animals in which physical factors decide whether or not their eggs develop parthenogenetically. In plant lice parthenogenesis is the rule only as long as the temperature is high or the plant has plenty of water. If we lower the temperature or let the plant dry out, sexual reproduction occurs. The drying out of the plant causes the tissues of the lice to lose water. The same factor, loss of water, makes the artificial parthenogenesis of Echinoderms and Chætopterus possible. In plant lice the effect is of the same kind, only in the opposite direction.

I have read somewhere the statement that Artemia salina is parthenogenetic, while Branchipus is not. Branchipus is a freshwater crustacean which, if raised in concentrated salt solutions (salt lakes), becomes smaller and undergoes some other changes. In that case it is called Artemia. If Artemia is parthenogenetic while Branchipus is not, it would mean that the *unfertilized* eggs of Branchipus cannot develop in fresh water, while they are able to develop in solutions of much higher osmotic pressure. This would be identical with our observation on the artificial parthenogenesis of Echinoderms and Chætopterus.

As I have mentioned in a former paper, O. Hertwig makes the statement that the unfertilized eggs of a number of marine animals which deposit their eggs in sea-water begin to develop after a number of hours, but do not develop beyond the first cleavage stages. Arbacia eggs reach the 2-cell stage in about 20 hours; the egg of Chætopterus may develop as far as 12 or 16 cells. According to Hertwig, not only the eggs of Annelids and Echinoderms, but also those of certain crustaceans show this peculiarity. I have mentioned in a former paper the observation made by Janosik that in the ovary of mammals occasionally eggs are found in the process of cell division. We shall make use of these facts in the next chapter.

I finally wish to say a few words concerning experiments published by Mr. Viguier of Algiers, Africa, who maintains that the eggs of Arbacia, Toxopneustes, and other sea-urchins are naturally parthenogenetic.[1] It would contradict neither my experiments nor my views if his statement were correct, as in all my papers I have assumed that these and many other (if not all) eggs have a tendency to develop parthenogenetically, and that it is only due to the constitu-

[1] VIGUIER: Comptes rendus de l'Académie des Sciences, Paris, July 2, 1900.

tion of the sea-water (or blood?) if they do not do so under natural conditions.[1] It might be that the constitution of the sea-water at Algiers differs from that of the rest of the world, and allows the eggs of the sea-urchin to develop parthenogenetically. The experiments of Mr. Viguier are, however, not of such a character as to make this probable. They are few in number, and he seems to have omitted no possibility which could further the contamination of his eggs by spermatozoa. He always handled males and females together, and opened males and females in the same experiment. No mention is made of a sterilization of his hands or instruments. Whenever males and females are in the same dish there is danger that the water may be full of spermatozoa, especially if the material is fresh. The sperm sticks to the surface of the females, and it is absolutely impossible to avoid fertilization of the eggs. To be sure, Viguier mentions a precaution he took, but this precaution shows that he is not familiar with the methods of sterilization or disinfection. He washed the females off in filtered sea-water. As everybody knows, the spermatozoa go through filter paper, and, in addition, sea-water does not remove the spermatozoa from the surface of the female, for the latter stick to solid bodies, as Dewitz has proved. In order to avoid this source of infection I washed the surface of the female several minutes in distilled water, or under a powerful stream of fresh water which kills the spermatozoa. I have in my former papers given a description of the precautions necessary in experiments on parthenogenesis.[2] These were by no means exaggerated if one wished to guard absolutely against contamination. I did not even succeed in excluding contamination by spermatozoa in my first Chætopterus experiment (see page 426), although my precautions were vastly superior to those taken by Viguier.

Another surprising fact in Viguier's paper is that he does not mention whether or not his unfertilized eggs had a membrane. In my researches on Arbacia I have considered the lack or presence of a membrane the most important criterion for deciding whether the development of the eggs is due to the entrance of a spermatozoon or to the osmotic or chemical treatment they have received. The fertilized eggs form a thick membrane, while the unfertilized eggs generally have no membrane (unless treated with certain salts in excessive quantities and for a long time). The cleavage of the

[1] See my preliminary note. This journal, 1899, iii, p. 135.
[2] LOEB: Science, 1900, xi, p. 612.

parthenogenetic egg that has no membrane differs so radically from that of the fertilized egg within a membrane, that it must arouse the interest or surprise of any morphologist. These differences are most noticeable during the first hours of the development. As soon as the egg approaches the blastula stage the membrane very often begins to disintegrate. I do not think that any experienced observer would have dared to publish the statement that the unfertilized eggs of Arbacia reach the pluteus stage, without having convinced himself that his "unfertilized" eggs had no membranes.[1]

Mr. Viguier makes the statement that he tried to repeat my experiments but was not able to confirm them. This does not surprise me, as he had not read my papers, and as he did not even know how my solutions had been prepared. My experiments have been repeated and confirmed by the following authors: Dr. C. Herbst (Naples), Professor E. B. Wilson (Columbia University), Dr. Hans Winkler (Tübingen), and Dr. S. Prowazek (Prague), and partly by Professor A. Giard (Paris). In addition they were re-peated with success by all the members of the class in physiology and embryology at Woods Holl last summer. As far as the statement is concerned that the unfertilized eggs of Arbacia or Strongylocentrotus are able to develop into plutei in normal sea-water, I can say that this is most certainly *not* the case at Woods Holl, in California (according to my own very numerous observa-tions), in Beaufort (North Carolina), and at Naples and other places on the Mediterranean, that have been visited by competent experimenters.

X. THE BEARING OF ARTIFICIAL PARTHENOGENESIS ON THE THE-ORY OF FERTILIZATION AND OF LIFE PHENOMENA IN GENERAL.

The general opinion concerning the rôle of the spermatozoon in the process of fertilization is that it acts as a *stimulus*, and that as such it starts the development of the egg. This statement is certainly wrong for those eggs in which we have been able to produce artificial parthenogenesis. For these eggs, like many others, begin to segment without any spermatozoon, if they are left long enough in normal sea-water. The only difference between these and the fertilized eggs is that the former begin to segment much later and

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[1] Viguier's paper has been criticised by A. Giard, Comptes rendus de la Société de Biologie, 1900, lii, p. 761.

their development stops in the early segmentation stages (2 to 16 cells at the most). The latter may be due to the fact that the egg dies before it has time to develop further.

If we consider the fact that the eggs show at least a beginning of a segmentation under "normal" conditions, the act of fertilization assumes a different aspect. The spermatozoon can no longer be considered *the cause* or *the stimulus* for the process of development, but merely an agency which *accelerates a process that is able to start without it*, only much more slowly. Substances that accelerate chemical or physical processes which would occur without them are called catalyzers (Ostwald). According to this definition we may assume that the *spermatozoon carries a catalytic substance* into the egg, *which accelerates the process that would start anyhow, but much more slowly.*

Through these facts and conceptions the phenomena of artificial parthenogenesis assume a different aspect. It would be wrong to say that *the K-ions are the stimulus* that causes the developmental process. *They merely act as catalyzers, accelerating a process that would otherwise proceed too slowly.* The loss of water on the part of the egg cell must have a similar effect, but possibly a less direct one. It may be that the loss of water alters the chemical processes in the egg in such a way as to give rise to the formation of a substance which acts catalytically.

Whether or not the catalytic substances introduced by the spermatozoon are identical with those employed in my experiments, I cannot say. I consider it probable that in the case of Chætopterus the natural fertilization is not brought about by K-ions, inasmuch as the normal development does not show the characteristics of a treatment of the eggs with K.

I have made a series of experiments with various enzymes to bring about the development of the unfertilized eggs of Arbacia, thus far without any results. The only enzyme that caused the egg to segment at all was papain. But I cannot be certain whether this was not due to some accidental constituent of the enzyme preparation used. The other enzymes were absolutely without effect. If we wish to find the active principle in the spermatozoon, we must make experiments in the direction of those begun by Winkler.[1]

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[1] WINKLER, H.: Nachrichten der königlichen Gesellschaft der Wissenschaften, Göttingen, 1900.

This author used extracts of the spermatozoon, and found that such extracts caused the eggs of sea-urchins to reach the 2-cell or 4-cell stage. As such a result can be brought about by slight alterations in the osmotic pressure or constitution of the sea-water, and as such alterations occurred in Winkler's experiments, I am not yet certain that his results were actually due to the substances extracted from the spermatozoon. But his experiments are certainly in the right direction.

The idea that the spermatozoon and the substances which cause parthenogenesis act only catalytically, has a great bearing upon the theory of life phenomena. It means that if we accelerate the processes of cell division in the mature egg (by specific catalyzers), the egg can live; but if these processes occur too slowly at the ordinary temperature (as is the case in the unfertilized egg in normal sea-water), the egg dies. The introduction of the catalytic substances which accelerate the processes of development saves the life of the egg. This may be made intelligible on the following assumption. Two kinds of processes are going on in the mature egg after it has left the ovary. The one leads to the formation of substances which kill the egg; the other leads to the formation of substances which allow growth and cell division, and are not poisonous. We may use as an illustration Pasteur's well-known experiments on the behavior of yeast cells in the presence and absence of atmospheric oxygen. In the presence of oxygen the yeast cells multiply on a sugar solution, while the zymase effect is comparatively small. In the absence of oxygen the multiplication of cells is limited or may stop, while the zymase effect becomes more prominent. The products of alcoholic fermentation are comparatively harmless for the yeast cell, and for this reason an increase in the fermentative activity of the cell does not cause the death of the yeast. I imagine that matters are similar in the mature egg cell after it has left the ovary, with this difference, perhaps, that the substances formed (by fermentation?) in the egg cell are more poisonous for the egg than the alcohol and the other products of fermentation are for the yeast. The process that causes the death of the egg cell and the one that causes cell division are at least partly antagonistic. .They are both inhibited by a low temperature, so that in this case death does not occur, although no cell division is possible. If we succeed in finding a substance which accelerates the process of cell division at the normal temperature, this will at the

same time lead to a suppression or a reduction of the antagonistic process that shortens life. In the case of the egg of Chætopterus a trace of K-ions acts as such a catalytic substance; possibly a trace of H-ions; and perhaps certain substances that are formed when the egg loses a certain amount of water. For the Echinoderm egg we know at present only the last factor. In addition there are the catalytic substances carried or produced by the spermatozoon (ions? enzymes?). But there are certainly other catalytic substances, as is proved by tumors and galls, in which the variety of structures corresponds to an almost equal variety of parasites.[1]

It is very important to realize that the introduction of catalytic substances into the egg does not prolong its life unless the egg has reached a critical point determined by two sets of conditions. The one is the maturity of the egg, the other the change of conditions connected with the egg leaving the ovary. As long as the egg is immature it lives without the introduction of these substances or the spermatozoon, and this may be true for the mature egg as long as it remains in the ovary. The fact that there is an age limit for the development of carcinoma may be a similar phenomenon. The catalytic substances which are given off by the cancer parasite may not be able to bring about cell division in the epithelial cells unless the latter have reached a critical point, which is at least partly determined by the age of the individual.

We generally consider development as a process which can only occur in one direction, or, in other words, is irreversible. But this is certainly not generally the case. I showed in a recent paper that the morphogenetic processes in hydroids are reversible. If the polyp of a Campanularia is brought in contact with a solid body, it is transformed into undifferentiated material and later into a stolon. If the same organ is brought in contact with sea-water, it gives rise to a polyp again.[2] The same may be done with Margelis and other hydroids. In Antennularia a change in the orientation of a branch with polyps will bring about the transformation of this material into a stolon. Between the two phases the material must pass through an

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[1] We do not need to assume a specific parasite for each kind of tumor. Teratomas may be explained on the basis of the parthenogenetic tendency of the mammalian egg in connection with some chemical change that furnishes the catalytic substance. But it is not impossible that even in benign tumors such as a teratoma the catalytic substance may be due to parasitic organisms.

[2] LOEB: This journal, 1900, iv, p. 178.

undifferentiated stage where it is neither polyp nor stolon. It will be the task to determine how far in the animal kingdom the developmental processes are found to be reversible. It is obvious that in a form with a reversible development death will not necessarily follow a certain stage of development (corresponding to senility in man).

It is not impossible that "natural" death is comparable to the situation which is present in the mature egg after it leaves the ovary. Nature has shown us the way by which at this critical point death can be avoided in the case of the egg.

THE THEORY OF PHOTOTACTIC RESPONSE.

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FROM the time of the earliest investigations on the heliotropism of plants and lower animal organisms, the literature of the subject has made frequent mention of "intensity of light" and "direction of ray." As early as 1853, Cohn,[1] after a study of Stephanosphæra and Chlamidococcus, concluded that the intensity of the light-stimulus alone governs the response to light; but later[2] he concluded that not intensity but direction of the ray of light is the important factor. Since that time nearly every investigator has favored one of the two views first indicated by Cohn. Thus Famintzin[3] in experiments with Chlamidomonas and Euglena, experiments which were imitated with oil-droplets in water by Sachs,[4] declared for intensity; whereas Strasburger, after confirming the observations of Famintzin, experimented further with various kinds of swarm-spores and reached the conclusion[5] that, "Swarm-spores which react to light move in the direction of the ray . . . and cannot move in any other direction." This, it is true, is not equivalent to saying that direction alone causes the movement. By more recent writers on the subject the two views have been more sharply separated, so that sensitiveness to changes in intensity of light is called *photopathy*, and to the direction of ray, *phototaxis*, an advance which, however, has not effected unity of opinion. Engelmann reported that the behavior of Bacterium photometricum shows a sensitiveness to intensity, but no reaction[6] which can decisively be called phototactic. Likewise Oltmanns,[7] after working with Volvox, wrote that differ-

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[1] COHN: Zeitschrift für wissenschaftliche Zoologie, 1853, iv. p. 111.

[2] COHN: Hedwigia, 1866, xi, p. 164.

[3] FAMINTZIN: Jahrbücher für wissenschaftliche Botanik, 1867, vi.

[4] SACHS: Flora, 1876, lix, pp. 241, 257, and 273.

[5] STRASBURGER: Jenaische Zeitschrift für Naturwissenschaft, 1878, N. F. v, p. 623.

[6] ENGELMANN: Archiv für die gesammte Physiologie, 1883, xxx, p. 121.

[7] OLTMANNS: Flora, 1892, lxxv, p. 183.

ences of intensity account for all the phenomena. On the other hand Loeb,[1] after an extensive study of heliotropism, concluded that " the intensity of the light affects the heliotropism of animals in this way, that only from a certain intensity of light onward do heliotropic movements result; and that the orientation of the animal in the direction of the rays is more precise as the intensity increases." Later Loeb[2] found a species of fresh-water planarian, Planaria torva, the individuals of which "are not oriented by the light-ray, therefore are not heliotropic, but . . . react very promptly to differences, or more exactly to changes, in light-intensity;" and since that time this author has maintained a distinction between heliotropic sensitiveness and sensitiveness to differences of intensity.[3] The most recent investigators[4] seem to agree more generally in recognizing both kinds of response. Thus Davenport[5] sums up a review of the subject as follows: " Two kinds of effects are produced by light; one by the direction of its ray — phototactic; the other by the difference in illumination of parts of the organism — photopathic." He states elsewhere,[6] " The best established of these phenomena is phototaxis."

When one looks at this subject for the first time it seems difficult to conceive how the direction and the intensity of the rays can operate apart. If light of a certain intensity falls on an organism from a certain direction, that side of the organism, it would seem, will be illuminated to that intensity, and the other side will lie in shadow; and one would expect the effect to be produced on the side where the light falls and in proportion to its intensity. But Davenport[7] explains, though other observers might have different interpretations, that " light acts directly either through difference in intensity on the two sides of the organism, or by the course the rays take through the organism." Phototaxis, then, depends solely on the course the rays take through the organism. How is this to be understood?

It was with a view of getting some insight into the potency of

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[1] LOEB: Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen, 1890, p. 109.

[2] LOEB: Archiv für die gesammte Physiologie, 1893, liv, p. 101.

[3] Cf. LOEB: Archiv für die gesammte Physiologie, 1897, lxvi, p. 439.

[4] DAVENPORT and CANNON: Journal of physiology, 1897, xxi, p. 22. YERKES: This journal, 1899, iii, p. 157. TOWLE: This journal, 1900, iii, p. 345.

[5] DAVENPORT: Experimental morphology, 1897, i, p. 211.

[6] DAVENPORT: Loc. cit., p. 203.

[7] DAVENPORT: Loc. cit., p. 210.

pure direction that the present writers undertook a set of experiments, in the first instance with Stentor cœruleus, and later with an unidentified species of Lynceus and several fresh-water planarians. It was hoped also, in case direction and intensity should be found beyond a doubt to be independent, to learn the conditions under which the two principles operate, that is, the relation between phototaxis and photopathy, a wholly untouched question. The experiments have confirmed much of the knowledge of heliotropism previously reported, and, as will be described later, have yielded new light on certain points. But the experimental study and a review of the literature on the subject have convinced us that the phenomena thus far reported do not demonstrate either that direction of ray and intensity of light operate separately, or that any distinction should be made between phototaxis and photopathy as independent forms of irritability. It is therefore the purpose of this paper, not to describe at length a series of experiments, but to consider systematically the facts of heliotropism, and to show how they may be consistently explained without recourse to the widely accepted assumption that direction and intensity act independently. The facts will be taken partly from reports of others and partly from original observations.

To commence with the simplest case of response to light, almost all motile organisms which are sensitive to light will move toward or from a source of light if free to do so. Thus, if a blue stentor be swimming at random in a glass dish, and light be admitted from one side, at first the animal seems to continue to circle about at random, yet, nevertheless, it is found to be actually moving away from the light; after a time it generally turns and swims definitely away from the source of illumination, till it reaches the farthest side of the dish. Lynceus usually swims, from the first, straight away from the light and does not digress at all. Other organisms have been found similarly to move toward light. None of the various explanations of the mechanism of the phototactic movements that have yet been suggested can be said to be altogether satisfactory. All, moreover, are extremely hypothetical, and an accumulation of observed facts is a great desideratum. The two most prominent observers who have attempted explanations are Loeb and Verworn, the former of whom professes to believe in direction and the latter in intensity. As a matter of fact the explanations offered by them, while not identical, are not in substance contradictory. These are best given

in the authors' own words. Loeb [1] writes of the phenomenon just described: " I pointed out five years ago that there exists a large number of animals which are *oriented* by light, in such a manner that they are forced to place their plane or axis of symmetry parallel to the rays of light. This can happen in two ways. The oral end can be turned toward or from the light. I called the animals in the first case positively, in the last case negatively, heliotropic." The explanation [2] runs as follows: " Suppose the light to strike one side of an animal. The immediate result is that changes are originated, which for the present we do not understand. *The outcome is a change in the tension of the muscles,* or elements which function as muscles. This change can be of two kinds: First, the light-stimulus can effect a preponderance in tension of the muscles of the illuminated side, or of those that turn the animal toward that side; and, secondly, it can produce the reverse effect, that is, a decrease of tension in these muscles and a greater tension of the muscles that turn the body toward the opposite side. I assume the first to happen in positively heliotropic, the second in negatively heliotropic animals. Thus the orientation of animals by light can be explained. Let SS_1 (Fig. 1) be parallel rays of light, and let a be the oral, b the aboral pole of a heliotropic animal. At the beginning of the experiment let the animal be moving in the straight line ba. The tension of the muscles that turn the animal

FIGURE 1. — Diagram illustrating LOEB's conception of phototactic movements. (From LOEB: *Loc. cit.* 1893, p. 87.)

to the right and to the left is therefore the same. But so soon as the light-rays SS_1 reach the right side, either (1) the tension of the muscles that turn the animal toward the light becomes greater, or (2) it becomes less; and, furthermore, in both cases the difference in tension of symmetrical muscles will be greater at the more sensitive oral pole a, than at the less sensitive aboral pole b. In the first case the animal is driven into the position ba_1, and next, according to the same supposition, it is forced to put its median plane in the direction of the light-rays; then it is positively helio-

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[1] LOEB: *Loc. cit.*, 1893, p. 81.   [2] LOEB: *Loc. cit.*, 1893, p. 86.

tropic. In the second case it is driven to the position ba₂, and is negatively heliotropic. As soon as its plane of symmetry comes to lie in the direction of the rays, all symmetrical points receive light of like intensity at a like angle, and so the animal is driven by the light neither to the right nor to the left, and progresses accordingly in the direction of the rays. As soon, however, as it is diverted from this direction by excitation from without or within, symmetrical points of the animal are again stimulated unequally by the light. The result is a corresponding change in the tension of symmetrical muscles, and the result of this, a return of the animal to the proper orientation."

Stated thus in general terms the hypothesis of Loeb seems not unreasonable, but it is not altogether clear how its author would apply it to the various cases of response to light. In view of this uncertainty and because the similar but more explicit hypothesis of Verworn can apparently be more readily applied to the facts, the latter will be provisionally adopted. It should be understood, however, that the design of this paper is not so much to compare rival hypotheses of the mechanism of the response or to establish one to the dethronement of the other, as to show the needlessness of the distinction commonly drawn between phototaxis and photopathy.
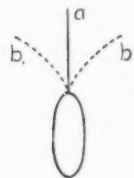
Fig. 2.—Scheme of the contraction of the flagellum of a flagellated organism. (From Verworn: *Loc. cit.*, 1899, p. 499.)

To Verworn it seems "wholly mystical" how the direction of the rays can affect the response, except as the incoming rays illumine one side of the organism, and leave the other side shaded, thus involving a different intensity of illumination on the two sides. In stating his hypothesis, Verworn[1] takes as the simplest case a flagellate with a single whip. "The flagellum of the Flagellata," he says. " is upon the anterior pole of the body and moves through the water in a screw-like path. For the sake of simplicity its motion may be considered as taking place in a single plane. It is then seen that it oscillates about the straight middle position (Fig. 2, a) by means of alternate rhythmic contractions toward the right (b) and toward the left (b₁); the swing out of the middle position (a) into one of the two extreme positions (b or b₁) represents the phase of contraction: the return from one of the extreme positions into the middle position.

[1] VERWORN : General physiology. English translation. 1899, p. 499.

the phase of expansion. The flagellum works, therefore, like an oar that is moved alternately to the right and to the left at the bow of a boat. It is evident that, while undisturbed and having equal conditions upon all sides, the infusorian body must move forward in a straight line, if the flagellum beats equally strongly toward the right and toward the left, *i.e.*, if contraction and expansion occur with equal rapidity toward the two sides. But if a contractile stimulus acts upon the flagellate suddenly from one side, and if the long axis of the body is not already turned in the direction of the stimulus with the posterior pole toward its source, such a position is assumed by means of a few strokes of the flagellum; for with every oblique or transverse position of the long axis the flagellum is stimulated to contract more strongly upon the side upon which the stimulus falls than upon the opposite side; it makes stronger strokes toward the former than toward the latter side, and the result is that the anterior part of the body is turned away
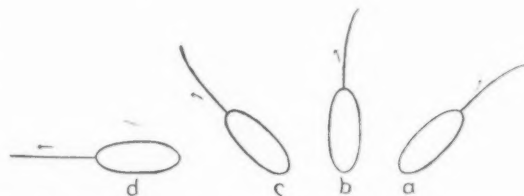
FIGURE 3. — Phototactic movements of a flagellated organism, according to VERWORN. (From VERWORN: *Loc. cit.*, 1899, p. 500.)

from the source of the stimulus (Fig. 3). Exactly the same relations exist here as in a boat moved by a single oar. The bow of the boat also turns toward the opposite side when the boat is propelled more strongly upon one side than the other. The unequal strength of the flagellar stroke in the two directions continues, and the anterior part of the body is turned constantly more away from the source of the stimulus, until the body has placed its long axis in the direction of the incident stimulus (Fig. 3, d). Then both sides of the flagellum become equally stimulated and the protist swims in a straight line, so long as the stimulus continues." "The same thing," Verworn[1] continues, " . . . occurs in ciliate Infusoria by means of the beat of numerous cilia. The movements of Paramœcium are analogous to the movements of a long boat possessing many oars. If all the oars upon the two sides move with exactly equal force, the boat moves straight forward; if

[1] VERWORN: *Loc. cit.*, p. 501

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the stroke of the oars is stronger upon one side than upon the other, the boat turns toward the opposite side. The same is true of the ciliary movement in Paramœcium. If the cilia beat with equal strength upon the two sides, the infusorian swims forward in a straight line; if, however, a contractile stimulus acts upon one side, so that the cilia upon that side are made to beat more strongly than upon the other, . . . the anterior end of the body must turn away from the source of the stimulus until its long axis is placed in the direction of the latter. The cilia then become stimulated equally upon corresponding points of the two sides of the body, and the cell swims forward in a straight direction away from the source of the stimulus." And finally, in general,[1] "the anterior pole of the body turns away from the source of the stimulus with excitation of contraction or depression of expansion upon one side; and toward the source of the stimulus with depression of contraction or excitation of expansion upon one side." The distinction between excitation of one phase of movement and depression of the opposite phase is not essential to this discussion, and will from now on be suppressed. Contraction will be used to signify either excitation of contraction or depression of expansion; expansion will mean the reverse.

Now Famintzin[2] very early observed that individuals of the genera Chlamidomonas and Euglena move toward diffuse light, but away from direct sunlight. That is, weak and strong intensities call out opposite responses. And this phenomenon has been observed of so many organisms that it is now generally admitted[3] "that the sense ($+$ or $-$) of response depends upon the intensity of the light," and[4] "that every organism has its optimum intensity of light for metabolism and response." That is, organisms move toward intensities weaker than their optimum and away from intensities stronger. Now since, according to the hypothesis just given, positive phototaxis results from excitation of the expansive phase of the cilia on the stimulated side, and negative phototaxis from excitation of the contractile phase, sub-optimal intensities must cause expansion and supra-optimal intensities must cause contraction.

And here may be pointed out a confirmation which this assumption finds in the direct observation of the orientation of the amœba

[1] VERWORN : *Loc. cit.*, p. 503.

[2] FAMINTZIN : Jahrbücher für wissenschaftliche Botanik. 1867, vi.

[3] DAVENPORT : Experimental morphology, 1897, i, p. 183.

[4] DAVENPORT : *Loc. cit.*, p. 199.

to light. To Davenport [1] belongs the credit of having shown, in experiments of great delicacy, that the amœba (Amœba proteus) is sensitive to light: and in fact that it responds negatively. Davenport does not say that he obtained any positive response. But when light was thrown on an amœba from one side, the pseudopodia were withdrawn on that side, and extended on the side farthest from the light, and the animal crept slowly away. According to Verworn's view of amœboid locomotion,[2] extension of pseudopodia is a phase of expansion due to decreased surface-tension at that point; withdrawal of pseudopodia is contraction due to increased surface-tension. Besides the *a priori* plausibility of this, it seems admirably supported by the well-known investigations of Berthold, Gad, Quincke, Kuhne, and Stahl. Whoever accepts this view can find in the withdrawal of pseudopodia under a supra-optimal light-stimulus, only a direct proof that supra-optimal intensities stimulate to contraction. It would be interesting to see whether sub-optimal intensities could be found that would induce extension of pseudopodia. It cannot be here objected that as the amœba is partially transparent, light should stimulate the farther side to the same extent as the nearer side. For if the light, in traversing the protoplasm, has its stimulative value not otherwise impaired, it is at least weaker by a certain function of the distance traversed. It will therefore in any case stimulate more strongly the side it reaches first.

Let us now consider the facts of response to light, and see whether they can be satisfactorily explained in accordance with this hypothesis of Verworn. We have selected four typical cases. These comprise not all phototactic phenomena, but those from which the idea has arisen that intensity of light and direction of its rays are independent in stimulation and productive of different responses.

The simplest case, in which an organism out of higher and lower intensities of light seeks to reach its optimum, has been described very clearly by Strasburger.[3] He worked with swarm-spores of Ulothrix and Hæmatococcus swimming in a drop of water. This drop was suspended in a moist chamber under the microscope, and received light from a window on one side. While the spores were

[1] DAVENPORT: *Loc. cit.*, p. 186

[2] VERWORN: General physiology, English translation. 1899, p 561.

[3] STRASBURGER: Jenaische Zeitschrift für Naturwissenschaft, 1878, N. F. v, p. 572.

near the window, they gathered farthest from the light, on the
" negative " side of the drop. As the preparation was slowly with-
drawn from the window to a darker part of the room, at first a few
spores, then ever increasing numbers, came over to the lighter side
of the drop, till finally all had crossed to the positive side. The re-
verse movement followed, as the preparation was again brought to
the window. Clearly now the light at the window was for all the
spores stronger than their optimum, since they tried to escape from
it into a weaker light ; and the light at the farthest side of the room
was weaker than their optimum, since all tried similarly to escape
from it into a stronger light. And the reason why not all the spores
left the negative side and swam across to the light at the same mo-
ment, is that they were attuned to slightly different optima, and each
spore changed sides only as its own region of optimal intensity was
crossed.

The mechanism of this response was outlined in the passage quoted
from Verworn, but a diagram will make the matter clearer. This
and the following figures will refer to a ciliated organism, say Para-
mœcium ; what is true of it is more obviously true of flagellate
species. Let us suppose at the outset that an organism is too near
the light : the stimulation is supra-optimal, phenomena of contrac-
tion result. If the first position is with the axis of the individual in
line with the light, the anterior end toward it (Fig. 4, a), the light
has no tendency to turn the individual about. Indeed the ordinary
forward movement is accentuated, by reason of the increased con-
traction of the forward cilia which catch the light (Cf. fig.). But
as no organism moves long in an absolutely straight line, this con-
dition does not last. At the first swerve to one side, the position b
is reached, where all the cilia on the lighted side contract more for-
cibly than those on the other, thus with every stroke turning the in-
dividual farther away from the light to the position c, and thence to
d. Here stimulation of the posterior cilia on the two sides is equal,
and tends simply to speed the retreat of the organism. Any random
divergence from the axial direction of d tends in the same way to
correct itself. The individual now progresses away from the too
intense light, till it reaches the region of optimal intensity. Here
the light seems to stimulate equally the phases of expansion and
contraction, so that no orientation results, and the organism swims
about within a certain region apparently at random.

Let us now suppose that the organism is too far from the light :

the stimulation is sub-optimal, the phase of expansion is augmented. The forward stroke of the cilia, it will be remembered, is conceived of as the expansive phase, the backward stroke as the contractile. If the first position of the organism is axially in line with the light, the posterior end toward it (Fig. 4, a_1), there is no movement tending to turn the body about, but the contractile stroke of the posterior cilia which catch the light is diminished, and the forward movement of the individual is impeded. Again the organism diverges by chance from the straight course into position b_1, and the backward stroke of the cilia on the light side is hindered, while the cilia of the shaded side work as effectively as before. Thus position c_1 is reached, and then d_1, where the organism faces the light and pro-
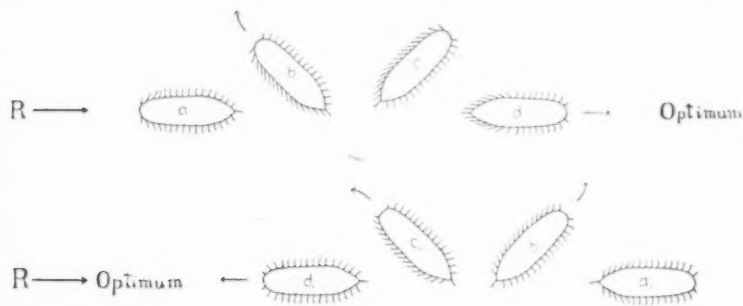
FIGURE 4. — Phototactic movements of a ciliated organism which is exposed unilaterally to light and then stimulated supra-optimally (above) or sub-optimally (below). R, rays of light.

gresses toward it, till the region of optimal intensity is reached. In this progression also random digressions from the straight course toward the light are self-corrective. It will be noticed here that as the organism progresses toward the light, the backward stroke of those anterior cilia which are more exposed to the light is lessened.

Thus the facts of this simple orientation, by which an organism seeks from both sides its optimal illumination, are reasonably and simply accounted for by Verworn's hypothesis.

A second case of response to light is that in which the organism is exposed to light from two or more sources. There has been some difference of opinion as to what the response under these conditions actually is. Loeb[1] has stated: " It scarcely needs to be remarked,

[1] LOEB: Archiv für die gesammte Physiologie, 1893. liv, p. 101.

that when rays of different directions fall simultaneously on an animal, and it is free to move in all directions, the more intense rays determine the direction of its movement." Also Davenport[1] inclines to this opinion, but only provisionally. He adds: "The point is worthy of detailed comparative study." Just this detailed study has since been contributed by Miss Towle in a report on some experiments with Cypridopsis vidua and Daphnia. Miss Towle[2] was led to the opposite conclusion from that of Loeb, namely: "The direction of movement . . . is determined (1) by the directions of all the impinging rays, and (2) by the relative value of these rays as forces acting upon the organism, *i. e.*, by their relative intensities. The resultant direction could be found by compounding all these forces if their direction and relative value were known." The somewhat intricate experiments of Miss Towle are to be considered in another place, but the concluding opinion, that if two rays of different intensities making an angle with each other, fall upon the organism, "each ray exerts its influence independently of the other," has been confirmed by ourselves.

In our experiments two 16-candle power electric lamps were placed symmetrically before an oblong glass dish, so that rays of equal intensity from the two lamps reached the dish and there met at an angle of about 40°. The form experimented with was Lynceus, which responds to this intensity negatively and with great precision. A single individual, on being dropped into the dish, started immediately away from the lights, and in a straight line which, if projected behind the animal, would have passed just midway between the two lamps. If one light was cut off, the animal, without halting, changed its course instantly and then moved straight away from the remaining lamp. If the first light was again turned on, the former direction was immediately resumed. Equally striking results were obtained with three lamps arranged as at three points of the compass; the crustaceans hurried unhesitatingly toward the fourth compass-point. Less marked, but still conclusive, were similar experiments on Planaria vortex.

Verworn's hypothesis would explain these facts somewhat as follows. Let there be two sources of light, and let an individual, supraoptimally stimulated, move by the first type of orientation away from one of the lights. Light from the other source falls on one side, augmenting the contractile phase, and so hastening the progress of

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[1] DAVENPORT: *Loc. cit.*, p. 208.   [2] TOWLE: This journal, 1900, iii, p. 365.

that side. Thus the organism turns also away from the second light, until, when equally stimulated on its two sides it progresses equally rapidly from both lights. That is, its course, if projected behind the animal, falls midway between the two sources of light. The explanation is analogous when the movement is toward the lights, or when there are more lights than two. As Miss Towle has shown, furthermore, if the lights are of unequal intensity, the resultant course of the animal is a function of both the position and the intensity of the two lights.

The third case of heliotropic phenomena to be considered is one that was first clearly observed by Oltmanns[1] in experiments with Volvox (globator and minor). It may essentially be stated as follows: If an organism is free to move at right angles to a parallel series of light rays artificially so modified that the illumination at one end of the course is bright and gradually shades off to darkness at the other end, the

FIGURE 5. — Observed phototactic movements of Stentor and Lynceus in aquaria seen from above and exposed to light that has passed through a prismatic screen.

organism again seeks its region of optimal illumination. Oltmanns realized these conditions practically as follows (Fig. 5). Parallel rays of light fell horizontally at right angles to the long axis of a narrow trough of water destined to receive the organisms. Between the trough and the incoming light there intervened a prismatic screen. Thin at one end, it there let almost all the light through; but becoming gradually thicker it gradually diminished the intensity of the rays till at the opposite end the screen was opaque and intercepted all the light. A volvox placed in the trough was thus free to

[1] OLTMANNS: Flora, 1892, lxxv, p. 183.

move from light to darkness and conversely, but was obliged to move always at right angles to the rays. Because of Oltmann's incautious conclusion [1] that " the determinative factor is not the direction in which the waves of light . . . are propagated, but rather the location of the optimum, that is the direction in which the intensity or concentration varies gradually toward the optimum," his experiments have been rather disparaged by those who believe in the efficacy of direction.[2] And it is to be granted that some peculiarities in his arrangement of light must have occasioned rather complicated shadows. But this much can hardly be denied, that his rays were perpendicular to the prism, and that his animals collected in the trough in places opposite to points of the prism which admitted light of a certain intensity. So unmistakable was this, that as the prism was brought opposite to different parts of the trough, the animals moved with the prism, keeping within their favorite illumination. Moreover, the careful quantitative work of Yerkes on Simocephalus vetulus Mueller, under identically the conditions described, puts this type of response beyond a doubt.

We have observed in some detail an analogous response with Stentor cœruleus and Lynceus. If numbers of blue stentors are put into a trough that is moderately illuminated under the conditions described above, the animals at first swim away from the light until they encounter the farther wall of the trough (Fig. 5). They then swim backward a little distance and start off in a new direction, as Jennings[3] has described for other species of Infusoria, some toward the light end of the trough and some toward the dark end. Soon they strike the wall again, and again start off in one direction or the other, and this series of movements is repeated many times. The preponderance of movement is toward the dark end, and in time by far the majority are found there swimming about. Lynceus shows this response rather more prettily, since it moves less at random. A single individual introduced at the light end of the trough near the front wall, moves in almost a straight line to the back wall (Fig. 5). This course is not quite at right angles to the two walls, but tends slightly toward the darker end. When Lynceus touches the wall it does not start backward like Stentor, but veers

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[1] OLTMANNS: *Loc. cit.* p. 263.

[2] Cf. DAVENPORT and CANNON : *Loc. cit.*, p. 24.　TOWLE: *Loc. cit.*, p. 361.

[3] JENNINGS : Biological lectures from the Marine Biological Laboratory, Woods Holl, 1899–1900, p. 105.

easily into the direction most nearly in line with its former course,
and goes toward the darker end of the trough, constantly keeping
its anterior pole close to the wall.

Let us now see whether Verworn's hypothesis will explain these
facts. In the first case let an organism be at the brighter end of the
trough, and let the stimulation be supra-optimal. The contractile
phase on the side toward the source of light is augmented. Let the
individual (Fig. 6, o) lie at first near the front wall of the trough,
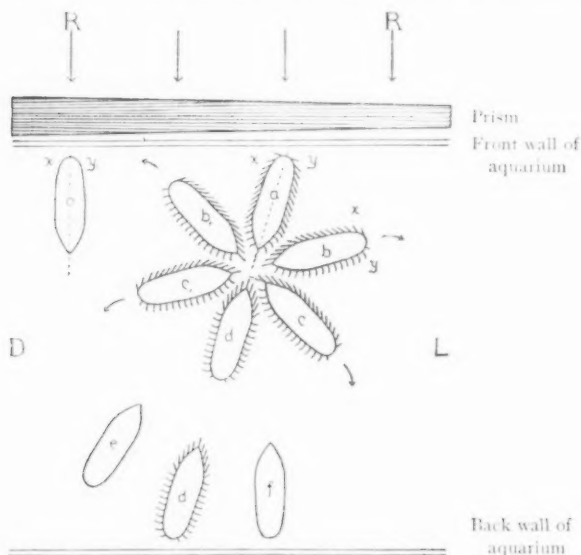
FIGURE 6.— Section of aquarium at the most brightly illuminated end, seen from above
and containing organisms exposed to light that has passed through a prismatic screen.
R, rays of light; L, lighter end, and D, darker end of aquarium.

with its axis perpendicular to the wall and to the axis of the prism.
It is clear that more light strikes side y of the organism than side x;
partly because y is opposite a thinner part of the prism, but more
especially because y receives considerable light which is reflected from
the water and sides of the trough at the well lighted end, L. The
fact that an appreciable quantity of light is thus reflected, is suffi-
ciently attested by Strasburger[1] and Miss Towle.[2] Thus this case is
like the second above mentioned, in which there are two sources of

[1] STRASBURGER : *Loc. cit.,* p. 588.   [2] TOWLE : *Loc. cit.,* p. 362.

light, and the animal so orients itself that they stimulate its two sides
equally. Now if the axis of the organism turns from position o so as
to point more toward the thin end of the prism, side x is more ex-
posed to the light transmitted by the prism, while side y is less ex-
posed to either the transmitted or the reflected light; in this way a
position will be possible in which the sides x and y are stimulated by
light equally. Let a (Fig. 6) be this position. Clearly here the light
exerts no moment to turn the organism about. But with the first
deviation from this direction the balance will be destroyed. Let us
then suppose the position b. Here all the cilia of the side x are
stimulated to contraction, and none of the cilia on the side y. Thus
the animal progresses to c, and thence to d. Similarly, if the second
position happened to be b_1, the movements would lead in turn to c_1
and d. Now positions d and a are clearly alike, inasmuch as in both
cases light stimulates the two sides of the organism equally. Clearly,
also, the axes in the two positions are parallel, so that at d the animal
is directed slightly toward the dark end of the trough — as was ob-
served of Lynceus before it reached the back wall (Fig. 5). It now
swims ahead, as we have seen in the cases of Lynceus and Stentor, till
it reaches the back wall of the trough. Since this is not encountered
at right angles, we should expect the organism to be simply veered
off in the direction D — as in fact we observed with Lynceus (Fig. 5).
We saw, however, that Stentor reacts thigmotactically, recoiling, and
then starting off again sometimes toward L but more often toward
D. In observing Paramœcium, Jennings[1] found that on stimulation
" the animal swims backwards, turns towards its own aboral side, then
swims forwards." A careful study of the thigmotactic response in
some flagellate and many ciliate species by the same author revealed
an analogous response as the usual one. For all species observed
" the direction of turning after a stimulus was towards a structurally
defined side, without regard to the nature and position of the source
of stimulus." According to Jennings, then, the organism is equally
free to take the new direction f or e (Fig. 6). The reason why the
stentors went eventually in greater numbers toward D, and thus ap-
peared oftener to choose e than f, is that such stentors as went to e
progressed farther toward D than those which went to f could pro-
gress toward L. These latter would soon strike the wall a second
time, now pretty nearly at right angles, and during the recoil the
light-stimuli would favor a return to d. It appears then amply pos-

[1] JENNINGS: *Loc. cit.*, p. 105.

sible that the circumstance that the organism encounters the wall of the trough at an acute angle, is sufficient to cause its further progress to be, in the long run, toward D. The angle that d makes with the wall of the trough in Fig. 6 is slight, but it is the angle at which Lynceus has been repeatedly observed actually to encounter the wall. And an individual seldom starts from d toward L. The average angle at which stentors cross the trough is less easy to ascertain, by reason of their many random movements. Miss Towle[1] describes the course taken by Cypridopsis and Daphnia under these conditions as "distinctly diagonal." It was probably more oblique, then, than the course of Lynceus as observed by us. Clearly the more oblique this course is, the stronger is the tendency toward D. In progressing toward D many species, of which Stentor is an example, sooner or later are driven again by the light to the wall of the trough, and the thigmotactic reaction as described has to be repeated. This circumstance retards the forward progress, but does not prevent it. Finally the organism reaches the region of its optimal intensity; the contractile stimulation of the light ceases; and further movements within this region are, from our point of view, made at random.

The movements of an individual placed at the dark end of the trough, sub-optimally stimulated, are to be explained, *mutatis mutandis*, by a description like the foregoing. Theoretically the most striking difference in the two cases would be that in approaching its optimum from the lighter end of the trough, the organism would collide with the wall farther from the light — as has been seen actually to be the case; whereas, in approaching from the region of sub-optimal intensity it would similarly collide with the wall nearer the light. For lack of positively heliotropic organisms this latter conjecture has not been verified.

Thus the response as observed is seen to be the one following most naturally from Verworn's hypothesis. When an individual reaches the wall of the trough the factor that favors its further progress in the direction of its optimal light-intensity, is the difference between the angle of collision (*i. e.*, the angle made by d with the wall) and a right angle; and this will be referred to as the essential feature in the following type of orientation.

We now come to the fourth and much discussed case, in which the rays of light fall obliquely through the prism (Fig. 7). Their source may be (A) above the thicker end, or (C) above the thinner

[1] TOWLE: *Loc. cit.*, p. 362.

end of the prism. In the former case an animal moving from the source of light comes into a region of higher intensity; in the latter case, into a region of lower intensity. Unquestionably under these circumstances organisms move with regard to the source of light, and not with regard to whether they are thereby brought into a region of weaker or stronger illumination. Thus, if an animal behind the prism is stimulated supra-optimally, it moves away from the source of light but into a region of brighter illumination. Curiously enough this response has been accounted crucial evidence that direction of ray sometimes operates alone, and the previous case of rays falling perpendicularly through the screen has been thought, on the other hand, to prove that intensity alone sometimes determines the response. We shall see that by our present hypothesis both phenomena can be explained in identically the same way.

Strasburger[1] devised these conditions. He laid a prismatic screen horizontally over his suspended droplet, and allowed light

FIGURE 7. — A, B, C, three aquaria seen from above and containing organisms exposed to light under different conditions. R, rays of light; L, lighter end, and D, darker end of aquaria.

[1] STRASBURGER: Jenaische Zeitschrift für Naturwissenschaft, 1878, N. F. v. p. 586.

to fall obliquely from over the thicker end of the prism. Thereupon the positively heliotropic swarm-spores of Botrydium swam toward the source of light into the darker side of the drop. If the prism was turned through 180°, the spores still moved toward the source of light, but this movement now brought them into brighter illumination. Since that time Davenport and Cannon[1] have done more detailed work under similar conditions. They found that "Daphnias known to be positively phototactic will move nearly uniformly towards the source of light, into a region of less intensity along the path of the incoming ray." Miss Towle also has experimented with a prismatic screen, on Cypridopsis and Daphnia, and by letting the light come obliquely through the prism from the side and not from above, she observed the actual course followed by the animals between the front and back walls more closely than it had been observed before. This was a diagonal course similar to that already observed by us in the above mentioned cases of Stentor and Lynceus.

If we apply Verworn's hypothesis to this case, we shall find that the explanation differs little from that of the previous case, where rays fell perpendicularly through the prism. There we found that the determining factor was the difference between the angle at which the animal crossed the trough toward the opposite wall and a right angle, that is, the direction of the axis of the animal in the position in which both its sides were stimulated by the light equally (a or d, Fig. 6). What in our present case will be this direction of equal bilateral stimulation? If, as in Fig. 7, A, an organism lies with its axis just in line with the rays, position o, as in the previous case (o, Fig. 6) its side y receives more light than x, because of the reflection from the lighter end of the trough, L. To make the stimulation on x and y equal, the axis must be placed more nearly at right angles with the prism, for then x receives more transmitted light from the prism, and y receives none but reflected light from L. Suppose a (Fig. 7) to be this position of equal bilateral stimulation. If the stimulation is supra-optimal, that is, contractile, the animal turns around to position d and from there veers toward L, exactly as in the second type of orientation. Thus, in evading the source of light, it moves into a brighter illumination. And, conversely, if the stimulation is sub-optimal, the animal will seek the source of light and thereby pass into darkness.

[1] Davenport and Cannon: Journal of physiology, 1897, xxi, p. 28.

Under these conditions, Miss Towle,[1] suspecting that the diagonal direction taken by the animals was the resultant of the direction of the light transmitted by the prism and that reflected from L, was able to vary the angle at which the rays met the prism until " the resultant movement of the two forces " was just at right angles with the axis of the prism. That is, d (Fig. 7) was the position of symmetrical stimulation. Here the animals swam at right angles to the prism and walls of the trough. Thus the author varied at will the direction that the animals took. Unfortunately her work was not arranged to show the further course of the animals after coming in contact with the front or back wall of the trough.

A glance at B (Fig. 7) will show that in the absence of a prism the position of symmetrical stimulation is in the direction of the light-rays, a; so that under supra-optimal stimulation the angle at which the animal encounters the back wall, even more than in case A, favors further progress away from the source of light. The reverse is the case for sub-optimal stimulation, that is, when the light (R, R) is weak. When, as in C (Fig. 7), the prism is turned through 180° from its position in A, clearly the axial direction for symmetrical stimulation inclines still more away from the light (here toward D); so that if in cases A and B the animal under supra-optimal stimulation moves away from the light, *a fortiori* it will do so here. Here indeed the increased illumination being on the side of the source, the case is really one in which the natural falling off in intensity away from the source of light is artificially exaggerated by interposition of a gradually darkening screen.

Thus, regardless of whether it moves into regions of higher or lower intensity, that is, regions where more or fewer rays strike it, the supra-optimally stimulated organism moves from the source, the sub-optimally stimulated one moves towards the source, of light. And we have seen that this response could have been anticipated from the simple assumptions of our hypothesis, namely, that light stimulates those parts of an organism on which it impinges, strong intensities (supra-optimal) stimulating to contraction, weak intensities (sub-optimal) to expansion. It is to be remembered that the course of the animals as described in these responses is not hypothetical, but has been observed: the diagonal movement in both directions between the front and back walls of the trough, by Miss Towle; and the movement of negatively responsive animals from the front obliquely

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[1] TOWLE : This journal, 1900. iii. p. 363.

to the back wall, their thigmotactic response, and their further move-
ment toward one or the other end of the trough, by ourselves. The
one thing not observed by us, for lack of material, is the corre-
sponding thigmotactic reaction of positively responsive organisms
against the front wall, and their further movement toward one or
the other end of the trough. The conditions for this observation
were realized by Yerkes, but being concerned with other features
of the response, that author did not consider this matter.

SUMMARY.

The foregoing argument has aimed to show that the assumption is
unnecessary and misleading, that the motor reactions of organisms
to stimulation by light are of two kinds, namely, reactions to the in-
tensity of light, and reactions to the direction of its rays. The phe-
nomena that have led to such an assumption can be satisfactorily
explained on the simpler theory that every ray of light impinging on
an organism stimulates at the point on which it falls, and in propor-
tion to its intensity, and that, as a result of this, organisms always
endeavor to seek their optimal intensity of illumination.

The direction of the rays has, in itself, no effect whatsoever on the
movements of organisms. It is true, however, that if the rays reach
the animal from a certain side, that side of the body is stimulated
more than the other, for the other side lies in its own shade. Hence
the controlling response is called out on the first side; but exactly
the same response would be produced if the same side of the organ-
ism could be similarly stimulated by rays coming from any other
direction whatsoever. The direction of the rays is therefore a second-
ary factor, operative only in so far as it determines what side of the
organism shall be stimulated. The same cilia stimulated by rays of
equal intensity would, regardless of the direction of the rays, always
yield the same response. Such statements, then, as those quoted in
the first part of this paper are erroneous, namely, that " Two kinds
of effects are produced by light; one by the direction of its ray —
phototactic; the other by the difference in illumination of parts of
the organism — photopathic; " or again, that " Light acts . . . by the
course the rays take through the organism."

Light acts in one way, that is, by its intensity. The light operates,
naturally, on the part of the animal which it reaches. The intensity
of the light determines the sense of the response, whether contrac-

tile or expansive; and the place of the response, the part of the body stimulated, determines the ultimate orientation of the animal.

By means of these two factors alone, the intensity of the light, and the side of the organism that the light reaches, this paper has aimed to explain all the facts of the phototactic response. It is freely admitted that the side on which the light falls is conditioned, in general, by the direction from which the rays come. But the strict distinction is insisted on, that the "direction of ray" in itself is absolutely immaterial. A given portion of an organism stimulated by a given intensity of light will respond, so far as is shown by the facts hitherto observed, by orienting the organism in a particular way. The facts do not show that the direction of the rays is otherwise effective than in determining on what part of the animal the light shall fall. There is no evidence that organisms respond to any other property of light than its intensity, and the distinction commonly made between phototaxis and photopathy as different forms of irritability is unwarranted.

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THE SPONTANEOUS SECRETION OF SALIVA AND THE ACTION OF ATROPINE.

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IN an earlier paper [1] I called attention to the spontaneous secretion of saliva from the submaxillary gland of the dog when, after its previous suppression for from ten to fifteen minutes, the circulation through the gland is re-established. During the past year a more extensive study of this phenomenon convinces me that it possesses decided interest for the theory of secretory nerves. In the paper cited I criticised that theory and expressed doubts of its correctness. Subsequent investigation has not changed my opinion except in unimportant particulars, and I hope in the immediate future to publish experiments justifying a reasonable scepticism.

It is generally believed at the present time that the secretion following stimulation of the chorda tympani nerve in the submaxillary gland is not due directly or indirectly to the coincident vasodilation, but to the action upon the gland cells of special secretory nerves. Howell,[2] in the recent edition of the American Text-Book of Physiology, states that the existence of secretory nerves is conclusively established; Langley,[3] in his elaborate discussion of salivary secretion in Schaefer's Text-Book, takes the same view, as do practically all physiologists. Nevertheless, while well aware that much may be said in favor of this hypothesis, it seems to me almost incredible that a theory of such importance should have been so generally accepted on such incomplete evidence. For it must be admitted that outside of the salivary and sweat glands of mammals there is very little evidence, worthy of the name, of the existence of any such nerves. We have only to consider the kidney, the

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[1] MATHEWS: The physiology of secretion. Annals of the New York Academy of Science, 1898, xi, pp. 343 and 357.

[2] HOWELL: An American text-book of physiology. Philadelphia, 1900, pp. 222 and 232.

[3] LANGLEY: Schaefer's text-book of physiology. London, 1898, p. 525.

liver, the stomach, to realize this. It is true that some of the facts of the secretion of these organs may be interpreted with more or less ease by that hypothesis, but it is not too much to say that had the salivary glands not been studied first no one would have come to the secretory-nerve hypothesis from a physiological study of any or all of these other glands. Nor is there any single phenomenon of their secretion which is not readily explicable without the assumption of secretory nerves; and this in spite of the close study these glands have had with the avowed purpose of discovering evidence of their existence. To what extraordinary lengths physiologists have been driven for evidence of the existence of such nerves in these glands may be seen in the otherwise fine paper of Pawlow on the pancreas. Pawlow[1] sums up his work with the statement: " Hence, finally, all these chief evidences of the existence of secretory nerves as distinct from the vasomotors hold also for the pancreas. These evidences are: 1, the independence of the secretory pressure from the blood pressure; 2, the paralysis of the secretory fibres by atropine; and 3, the increase upon nerve stimulation not only in the amount but also in the concentration of the secretion."

We thus see one of the leaders of physiology avowing his acceptance of a far-reaching theory upon the basis of three facts, one of which has no bearing whatever on the matter, and the other two are, to say the least, readily susceptible of other explanation. For whether secretory nerves, properly speaking, be hereafter shown to exist in the pancreas or not, the utter insufficiency of this evidence of their existence must be clear to any unprejudiced mind. Of these three facts the first is manifestly quite beside the mark. It has no bearing whatever on the question of the existence of secretory nerves. It shows only that secretion is not in this instance a filtration. It is undoubtedly to be explained by the difference in osmotic pressure between the gland contents and the blood, and is dependent on the resistance of the gland to back filtration. The second fact, the action of atropine, is in no better case so far as it concerns the pancreas. It would seem superfluous to have to call the attention of so able a physiologist as Pawlow to the obvious and well known fact that the sole evidence we have that atropine paralyzes the secretory nerve ends, and not the gland cells, is derived entirely from the salivary glands and almost completely from the

[1] PAWLOW: Archiv für Physiologie, Supplement-Band, 1893, p. 191.

submaxillary gland of the dog. It would be superfluous were the fact not so generally ignored.

The proof that atropine paralyzes the nerve ends and not the gland cells is the fact that the drug paralyzes the chorda but not the sympathetic in the dog's submaxillary. For if the sympathetic is not paralyzed the gland cells must still be active. Atropine must, therefore, have acted on something else. What more likely than upon the secretory nerve ends? The weakness of this argument even for the salivary glands will be sufficiently apparent; for two unproved assumptions are involved, first, that the sympathetic causes its secretion by action on the gland cells, and, second, that atropine does not act on anything else than these nerves. Both of these assumptions I have already shown to be probably incorrect.[1] But quite apart from the condition of things in the salivary glands, it is obviously improper to carry over bodily and without question to the pancreas these conclusions derived from the drug's action elsewhere. There is no evidence at all that in the pancreas the drug paralyzes the nerve ends rather than the gland cells. For in the pancreas there is no other secretory nerve not paralyzed by the drug and it is quite conceivable that the drug might act on the cells of the pancreas, but not on those of the submaxillary. Until it shall have been shown that atropine does not paralyze the cells of the pancreas the action of atropine on the pancreas is worth little or nothing as establishing the existence of secretory nerves. In a forthcoming paper I hope to show that atropine does act on the pancreas cells. Pawlow's third point is also, in my opinion at least, of doubtful value, since a variety of explanations may be given of this observation. I hope to consider this point more closely in the near future.

To return to the salivary glands, there are three main evidences of the existence of secretory nerves here: first, the action of atropine; second, the post-mortem chorda secretion, and, third, the increase in concentration coincident with an increase in the rate of secretion.

In regard to the post-mortem chorda secretion it must be remembered that a similar secretion occurs only in the sweat glands, the skin glands of Amphibia, and the salivary and lachrymal glands. We get no secretion in the absence of blood supply in the pancreas, kidney, liver, stomach, or any other vertebrate glands. This fact gains

[1] MATHEWS: *Loc. cit.*, pp. 303 and 349.

significance if the structure of these glands is considered. The sweat, sebaceous, lachrymal and amphibian skin glands are all surrounded by contractile sheaths. As regards the salivary glands, which are epiblastic, the presence of such a contractile sheath, which I foretold from physiological evidence,[1] has been shown recently by Kolossow.[2] The pancreas, kidney, liver, and stomach have no such sheaths. A secretion without blood supply is obtained then only in those glands which have a contractile sheath, which in many cases at least has been shown to be innervated by the so-called secretory nerves.[3] Is this fact without significance, — to be passed over in silence? The recent experiments of Bunch[4] also indicate that when the chorda is stimulated a contraction of this sheath takes place similar to that following stimulation of the sympathetic. How otherwise is the fact, discovered by Bunch, to be explained, that in stimulation of the chorda the gland actually decreases in volume in spite of the increased blood supply? Bunch's full paper has not yet appeared, so that it is impossible to know how he may explain it, but he seems in his preliminary communication to regard the diminution in bulk as due to the decrease in size of the cells brought about by the discharge of their secretion. This explanation cannot possibly be the right one unless it be assumed that the alveoli are under high elastic tension, and that it is easier for that tension to force the saliva from the ducts than from the cells. For it is clear that the discharge of substance from the cell into the gland lumen will increase the bulk of the saliva in the lumen to just the extent that the cell diminishes in bulk. There will hence be no pressure generated and no *vis a tergo* to drive the secretion from the ducts and alveoli. It seems to me that Bunch's experiments render Unna's[5] old theory, that the chorda causes secretion by action of the nerve on some contractile sheath, highly probable.

It is assumed that the submaxillary gland will not secrete after vasodilation alone, but that irritation of the secretory nerves is necessary. That this opinion may be incorrect, in the case of the

[1] MATHEWS: *Loc. cit.*, p. 328.

[2] KOLOSSOW: Archiv für mikroscopische Anatomie, 1898, lii, p. 1.

[3] DRASCH: Archiv für Physiologie, 1889, p. 127. ENGELMANN: Archiv für die gesammte Physiologie, 1872, v, p. 498. JOSEPH: Archiv für Physiologie, 1891, p. 81.

[4] BUNCH: Journal of physiology, 1900, xxv, p. xii; British medical journal, 1900, p. 842.

[5] UNNA: Centralblatt für die medicinischen Wissenschaften, 1881, p. 258.

dog's submaxillary at least, I believe the following experiments indicate. This opinion rests chiefly on the action of atropine. Atropine permits vasodilation but no secretion, hence Heidenhain inferred that vasodilation could not cause secretion. While this inference may be true, he has by no means shown it to be so, for he can hardly argue that the same conditions hold in the normal as in the poisoned gland.[1] It is quite possible that vasodilation in the normal gland by suddenly increasing the oxygen and food supply of the previously anæmic organ might lead to chemical changes in the cell protoplasm indirectly increasing its osmotic pressure and causing it to secrete, whereas in the atropinized gland these conditions may be unable to bring about secretion.

Several facts indeed indicate that glands can secrete quite independently of secretory nerve impulses, and as a result of vasodilation. The secretions of all or nearly all invertebrate glands are constant, and appear to be carried out quite independently of nerve action. In the mammals, the so-called paralytic secretions, which may last for days in the absence of nerve impulses, are difficult to explain unless secretion can be carried on by the gland cells under variations of blood supply. We have, besides, the secretion of sweat which occurs in the horse on division of the cervical sympathetic. Here as a concomitant of vasodilation a profuse and constant sweating occurs. The other explanation suggested by Arloing,[2] that the nerve carries inhibitory secretory sweat fibres, shows to what lengths the secretory nerve hypothesis has carried us. The fact, too, that all the great secretory nerves are vasodilator nerves is so well known that it has seemed to lose its significance. The chorda tympani, Jacobson's nerve, the lingual, the vagus for the pancreas and stomach, are some of these. Consider for a moment the extraordinary condition of the pancreas. If the peripheral freshly divided vagus nerve is stimulated the pancreas does not secrete. This means either that secretion in the pancreas is absolutely dependent on vasodilation or that the nerve carries secretory-inhibitory, as well as excitatory fibres. The last possibility may be discarded at once as altogether improbable. The first supposition is

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[1] LEVY (Verhandlungen der physiologischen Gesellschaft zu Berlin, VI Sitzung, Archiv für Physiologie, pp. 155 *et seq.*) has already expressed similar views upon the action of atropine.

[2] ARLOING: Archives de physiologie normale et pathologique, 1891, xxiii, p. 249.

probably the true explanation, for if the vagus be allowed to degener-
ate for several days, a proceeding necessary to give the nerve a
dilator action, secretion ensues. Finally Levy[1] has observed, in the
cat's foot, that if the blood be cut off for three hours and a half or
more, on readmitting it the sweat glands spontaneously secrete.
Since it is well known that in such conditions the blood-vessels are
dilated, we have another case of vasodilation produced without nerve
stimulation, and secretion accompanying it.

I have found that the dog's submaxillary reacts like the sweat-
glands. If the blood is cut off from the gland for 7–35 minutes and
then readmitted, after a pause of a minute or two the gland begins to
secrete and secretes rapidly for several minutes in the complete ab-
sence of nerve stimulation.

The method of procedure, with the exception of the two last ex-
periments in which the gland was perfused with defibrinated blood,
consisted in cutting the vagus-sympathetic in the neck and the
chorda-lingual, then exposing the gland by cutting the skin over it,
and dividing and extirpating the digastric muscle and tying all arte-
ries except the one entering the hilus. The operation is long and
tedious, and owing to the variation in position of blood-vessels often
unsatisfactory. I have found it necessary to ligature practically all
arteries, for if any are left, except the very smallest, the result will be
uncertain, the gland sometimes secreting on readmitting the blood,
sometimes not. A number of the exceptions which appear in the
following experiments are due to a failure to ligature all vessels or to
the kinking of the hilus artery owing to the abnormal position of the
gland after its dissection. The veins were left intact. It is easier
and more certain to perfuse the gland with defibrinated blood after
the death of the dog, for the artificial circulation is then under com-
plete control.

There are several arteries entering the gland, one coming in at the
hilus and one entering the middle of the dorsal surface of the gland.
These are the larger and more obvious. There are besides small
vessels occasionally entering from the gland tunic at various points,
and particularly a very small vessel coming near the lingual nerve
and following the chorda in. Several branches, also given off from
the external maxillary artery, enter the sublingual gland and pass
thence to the submaxillary. These little vessels I have found most diffi-
cult to exclude. In the perfusion experiments the cannula was intro-

[1] Levy: *Loc. cit.*

duced into the external maxillary artery, beyond the point of origin of the gland artery. The external maxillary artery was then tied near its origin from the carotid and after a pause of several minutes and the death of the animal by bleeding, perfusion was begun. No salt solution was added to the defibrinated dog's blood. Large dogs were used; morphine sulphate was given subcutaneously before the operation, and ether was given during the operation.

In the following tables C or S in the middle column indicates that at these times the chorda or the sympathetic was stimulated. The small figures immediately after indicate the number of seconds of stimulation. Where no letter is written no nerve was stimulated.

Experiment XIII, Oct. 24. — Dog. Weight 25 lbs. Usual operation. 2 c.c. 3 per cent morphine at 9 A.M. 10 mm. = 0.038 c.c.

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
12.42.00	C_{10}	59	1.26.00	C_{20}	3
44.00		Clamped artery.	28.00		Unclamped.
44.00—45.00		20	28.00—29.00		10
46.00		17	29.00—30.00		95
48.00—49.00		2	30.00—31.00		165
49.00	C_{20}	23	31.00—32.00		114
50.30—51.00		5	32.00—33.00		61
51.00	C_{30}	20	33.00	C_{10}	105
53.00	C_{30}	11	34.30		Clamped.
56.00	C_{30}	3	34.30—35.00		10
1.00.00	S_{30}	0	35.00—36.00		1
03.00		Unclamped.	36.00—48.00		0
03.00—04.00		3	49.00		Unclamped.
04.00—05.00		111	49.00—50.00		19
05.30—06.00		260	50.00—51.00		45
06.00—07.00		175	51.00—52.00		125
08.00—10.00		145	52.00—53.00		110
10.00—12.00		70	54.00	C_{10}	82
12.00—13.00		35	55.00—55.30		30
13.30	C_{10}	45	56.00	C_{10}	140
14.00—15.00		25	57.00—58.00		70
15.00	C_{10}	58	58.20		Clamped.
16.00—17.20		25	58.20—58.40		8
17.20		Clamped.	58.40—59.00		0
17.20—18.00		4	59.00—60.00		0
18.00—22.00		0	2.00.00—01.00		5
22.00	S_{30}	0	01.00—02.00		2
22.30	C_{20}	12	02.00—04.00	C_{20}	4
23.00—24.00		1	04.00—05.00		3
24.00	C_{20}	7	05.00	C_{20}	3
25.00	C_{20}	3	07.00—09.00		0

Experiment XIII — (continued).

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
2.09.00		Unclamped.	2.31.00–32.00		35
09.00–10.00		13	32.00–33.00	C_{10}	240
10.00–11.00		150	33.30–34.00		115
11.00–12.00		175	34.00–35.00		100
12.00–12.40		40	35.10		Clamped.
12.40–		Clamped.	35.10–35.40		30
12.40–13.00		20	35.40–37.00		10
13.00–13.10		1	37.00		Unclamped.
13.10–14.00		0	37.00–38.00		50
14.00–15.00	C_{20}	16	38.00–39.00		50
15.00–15.30		0	39.10		Clamped.
15.30		Unclamped.	39.10–39.40		5
15.30–16.00		15	40.00		2
16.00–17.00		48	40.00–41.00		0
17.00–18.00		100	41.00–43.00		2
18.00–18.20		25	43.00		Unclamped.
18.20		Clamped.	43.00–44.00		2
18.20–18.40		15	44.00–45.00		38
18.40–19.00		0	45.30		1 c.c. 0.5 per cent atropine
19.00–20.00		0			sulphate in femoral vein.
20.00–21.00	C_{10}	18	45.00–46.00		40
21.15		Unclamped.	46.00–46.45		15
21.15–22.00		0	46.45–47.00		6
22.00–23.00		130	47.00–48.00	C_{20}	2
23.00–24.00		115	48.00	C_{20}	0
24.00–25.00		34	53.00	S_{20}	30
25.00–28.00		51	53.00–56.00		0
28.00–30.00		37	56.00	C_{20}	0 End.
30.00–31.00		45			

Experiment XIV, Oct. 26. — Dog. Weight 30 lbs. Operation as usual. 2.5 c.c. 3 per cent morphine at 9 A.M.

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
11.48.00	C_{10}	55	12.14.30–14.45		35
49.00	S_{30}	5	15.00–15.30		145
58.00		Clamped.	15.40–16.00		80
59.00–64.00		0	16.00–16.40		85
12.05.00	C_{20}	18	17.30–18.30		32
06.00–07.00		17	18.30–20.00		33
07.00–08.00		7	20.00–23.00		10
08.00	C_{20}	8	23.00–27.00		20
09.00–10.00		4	27.00–30.00		17
11.00	S_{30}	12	30.00	C_{10}	63
13.00	C_{20}	16	31.00–32.00		7
14.00		Unclamped.	32.00		Clamped.
14.00–14.30		19	32.00–33.00		3

Experiment XIV — (continued).

Time. h. m. s.　m. s.		Secretion in mm.	Time. h. m. s.　m. s.		Secretion in mm.
12.33.00	C_{10}	65	1.32.00–32.30		6
34.00	C_{10}	15	32.30–33.00		11
35.00	C_{20}	43	33.00–34.00		14
36.00–37.00		10	34.00–35.00		23
38.00	C_{20}	25	35.00–36.00		44
39.00	C_{20}	22	36.00–37.00		32
40.00	C_{20}	13	37.00–38.00		24
41.00	C_{20}	10	38.00	C_{10}	64
42.00–43.00		5	40.00		Clamped.
43.00	C_{15}	8	40.00–40.30		6
44.00–45.00		6	40.30–41.00		5
45.00	C_{15}	6	41.00–43.00		0
46.00–47.00		6	43.00–48.00		30
47.00	C_{20}	4	48.00–52.00		10
48.00	S_{20}	10	53.00		Cut vein, artery unclamped.
49.00		Unclamped.	53.00–53.10		20
49.00–49.10		5			Blood spurts from vein.
49.10–49.30		10			
49.30–50.00		10	54.00–55.00		40
50.00–51.00		30	55.00–56.00		70
51.00–52.00		15	56.00–57.00		50
52.00	C_{10}	65	57.00	C_{25}	55
Stimulate C several times. Secretes 5–9 c.c. Freed gland completely.			58.00		Clamped.
1.08.40		Clamped.	2.00.00–01.00		10
08.40–10.00		9	01.00	C_{10}	45
10.00	C_{25}	39	05.00	C_{20}	10
11.00–12.00		2	06.00–07.00		4
12.00	C_{25}	38	07.00–08.00		10
13.00–14.00		4	08.00	C_{20}	12
14.00	C_{25}	20	09.00–10.00		2
15.00	C_{20}	17	10.00	C_{20}	0
16.00–17.00		3	11.00	S_{30}	11
17.00	C_{45}	25	12.30		Unclamped.
18.00–19.00		5	12.30–13.00		5
19.00–20.00		24	13.00–14.00		10
20.00		Second clamp on artery.	14.00–15.00		25
20.00	C_{20}	23	15.00–16.00		85
21.00–24.00		8	17.00–17.50		75
24.00	C_{20}	12	17.50		Clamped.
26.00	C_{20}	5	17.50–18.30		20
27.00	C_{20}	3	18.30–19.00		5
28.00–29.00		4	19.00–20.00		0
29.00	S_{30}	6	20.00–24.00		6
30.00	S_{30}	0	24.00	C_{20}	3
31.00–32.00		0	26.00	S_{30}	5
32.00		Unclamped.	28.40		Unclamped.
			28.40–29.40		8

Experiment XIV — (continued).

Time. h. m. s. m. s.	Secretion in mm.
2.30.00–31.30	35
31.30–32.30	165
33.00–34.00	100
35.30	Clamped.
35.30–35.40	2
35.40–37.30	24
37.30	Unclamped.
37.30–38.00	4
38.00–39.00	20

Time. h. m. s. m. s.	Secretion in mm.
2.39.00–40.00	26
40.00–41.00	55
47.00–48.00	33
1 c.c. 0.5 per cent atropine sulphate left jugular.	
48.00–49.00	2
49.00–50.00	0
	No more.

Experiment XVI, Oct. 31. — Dog. Weight 17 lbs. 1.5 c.c. 3 per cent morphine subcutaneous at 9 A.M. First gland a failure owing to abnormal artery distribution. At 2 P.M. operated on right gland.

Time. h. m. s. m. s.		Secretion in mm.
2.15.30		Clamped.
16.00	C_{10}	10
17.00	C_{20}	10
18.00–19.00		0
19.00	C_{20}	10
20.00	C_{20}	5
21.00–22.00		0
22.00	C_{20}	2
23.00	C_{20}	0
24.00	S_{30}	0
25.00		Unclamped.
25.00–26.00		28
26.00–26.10		25
26.25–27.00		100
27.00–27.25		50
27.30–28.00		40
28.00–29.00		40
31.00	C_{10}	30
32.00	S_{20}	15
33.00–34.00		4
35.10		Clamped.
35.10–37.00		7
37.00	C_{20}	3
39.00	C_{20}	2
40.00	C_{20}	0
41.00	C_{20}	2
42.00	S_{20}	0
43.00		Unclamped.
43.00–43.30		2
43.30–45.00		3

Time. h. m. s. m. s.		Secretion in mm.
2.45.00–46.00		0
46.00–48.00		0
49.00	C_{10}	15
54.00		Clamped.
54.00–63.00		27
3.03.00	C_{20}	0
04.00	S_{30}	0
05.00		Unclamped.
05.00–05.45		Begins.
06.00–07.00		55
07.00–08.00		60
08.00–09.00		27
09.00–10.00		8
11.00	C_{10}	0
13.30		Clamped.
18.40	C_{20}	0
19.00–20.00		5
20.00	C_{20}	5
21.00–22.00		2
22.00	S_{30}	3
23.00		Unclamped.
23.00–23.30		0
23.30–24.00		5
24.00–25.00		10
25.00–26.00		30
26.00–27.00		16
1 c.c. 0.5 per cent atropine into crural vein.		
27.00–28.00		1
		No more.

Experiment XVII. — Bitch. Weight 80 lbs. 6 c.c. morphine sulphate, subcutaneous. 8 A.M.

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
11.25.00	C_{05}	80	12.14.30–15.00		87
47.00		Clamped.	15.00–16.00		80
47.00–48.00		20	18.00	C_{20}	65
48.00–49.00		20	19.10		Clamped.
49.00	C_{10}	80	19.00–21.00		5
50.00–51.00		17	21.00	C_{20}	75
51.00–52.00		7	23.00	C_{20}	0
52.00	C_{10}	6	26.00	C_{20}	0
53.00	C_{20}	7	26.30	S_{20}	0
54.00–55.00		10	27.00		Unclamped.
55.00	C_{10}	0	27.00–28.00		20
56.00–58.00		0	28.00–29.00		40
58.00	S_{20}	0	29.00–29.35		80
59.00		Unclamped.	29.35–30.00		50
59.00–60.00		5	30.00–31.00		15
12.00.00–01.00		40	31.30		Clamped.
01.20–02.00		75	31.30–32.00		20
02.00–02.40		90	32.00–34.00		0
03.10–04.00		75	34.00–35.00		5
04.00–05.00		90	35.00	C_{30}	10
05.10		Clamped.	36.00–40.00		
05.30–06.00		5	40.00		Unclamped.
06.00–07.00		6	40.00–41.00		10
07.00–10.00		3	41.00–42.00		5
10.00	C_{20}	11	42.00–43.00		30
11.00–12.00		0	43.00–44.00		42
12.00		Unclamped.	Atropine in jugular vein, 1 c.c		
12.00–12.30		3	0.5 per cent.		
12.30–13.00		7	44.15–45.00		7
13.00–14.00		90	45.00–46.00		0
14.00–14.15		30	46.00	C_{20}	0

Experiment XXI. — Dog. 30 lbs. 3 c.c. 3 per cent morphine sulphate at 9 A.M.

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
10.45.00	C_{20}		10.54.00–55.00		2
46.00–47.00		50	55.00	C_{10}	38
47.00–48.30		60	56.00–57.00		10
48.30		Clamped.	11.00.00	C_{10}	6
48.30–49.00		5	01.00	C_{10}	12
49.00–50.00		0	04.00	S_{20}	7
50.00–51.00		1	05.00		Unclamped.
51.00–52.00		2	05.00–05.30		5
52.00–53.00		7	05.30–06.00		83
53.00–54.00		5	06.20–06.50		130

Experiment XXI — (continued).

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
11.07.30	S_{45}	65 Stops.	11.31.00–32.00		28
08.45–09.00		25	32.00–33.00		18
09.00–09.20		10	33.00–34.00		12
09.30–10.00		120	34.00–35.00		7
11.00–11.30		80	35.00–36.00		5
11.30		Clamped.	36.00–37.00		5
11.30–12.00		15	37.00–38.00		7
12.00–12.30		15	38.00	C_{10}	118
12.30–13.00		2	39.00–40.00		40
13.00–14.00		1	40.00–41.00		47
14.00–15.00		1	41.00	S_{25}	68
16.00	C_{29}	20	43.00–47.00		99
17.00	C_{29}	5	47.00–48.00		17
20.00	S_{30}	4	48.00–49.20		25
22.00		Unclamped.	49.20		Clamped.
22.00–23.00		6	49.20–49.40		1
23.00–24.00		45	49.40–50.00		0
24.00–25.00		60	50.00–55.00		4
25.00–25.25		20	55.00–56.00		1
25.30–26.30		55	56.15		Unclamped.
26.30		Clamped.	No secretion.		Dissected right gland.
26.30–27.00		5	3.31.00		Clamped.
27.00–28.00		4	31.00–37.00		20
28.00–28.30		0	40.00	C_{29}	37
28.30		Unclamped.	41.15		Unclamped
28.30–29.00		11	41.15–42.00		57
29.00–30.00		65	42.00–44.00		80
30.00–30.15		20	44.00–44.10		13 End.
30.30–31.00		30			

Experiment XXII. — Bitch.   Weight 25 lbs.   2 c.c. 3 per cent morphine sulphate at 8.45 A.M.

Time. h. m. s. m. s.		Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
11.46.20		Clamped.	12.03.00–04.00		20
46.20–51.00		—6	04.00–05.00		30
51.00	C_{29}	25	05.00–06.00		17
53.00	C_{29}	13	06.00–07.00		7
54.00–55.00		2	07.00–08.00		2
55.00	C_{29}	6	09.00	C_{10}	29
56.00	C_{29}	2	14.00		Both S and C non-active.
57.00	S_{30}	0			Cut both vein and artery on
58.00–59.00		0			upper surface of gland. Gland
12.00.00		Unclamped.			begins to secrete spontane-
00.00–01.00		2			ously.
01.00–02.00		2	18.00	C_{10}	38
02.00–03.00		6	19.00–20.00		2

Experiment XXII — (continued).

Time. h. m. s.　m. s.		Secretion in mm.	Time. h. m. s.　m. s.		Secretion in mm.
12 20.00	C_{10}	23	12.43.00—44.00		7
		Artery kinked.	44.00—45.00		28
28.00		Adjust gland.	45.00—46.00		23
28.00—28.30		5	47.00—48.30		25
28.30—28.50		40	48.30—56.00		31
29.00—30.00		135	56.00—57.00		1
30.00—30.15		25	1.01.45		Readjust gland.
30.00		Clamped.	01.45—02.00		35
30.30—31.00		20	02.00—03.00		108
31.00—31.30		5	03.00—04.00		55
35.30	C_{10}	15	04.00		Clamped.
38.30	C_{20}	0	04.00—04.30		5
39.30	S_{20}	0	05.00	C_{10}	40
42.00		Unclamped.			End.
42.00—43.00		5			

Experiment XXIII. — Dog. Weight 90 lbs. 7 c.c. 3 per cent morphine sulphate at 9 A.M.

Time. h. m. s.　m. s.		Secretion in mm.	Time. h. m. s.　m. s.		Secretion in mm.
12.58.00		Clamped.	1.39.00		Unclamped.
1.04.00	C_{20}	11			No secretion.
07.00	C_{20}	0	47.00	C_{10}	58
10.00		Unclamped.	48.00—49.00		80
10.00—10.30		13	49.00—50.00		40
10.30—11.00		7	50.00—51.00		30
11.00—11.20		18	51.00—52.00		35
11.30—12.30		130	52.30		Clamped.
12.30—12.40		30	53.30—53.00		5
12.45—13.00		60	53.00—57.00		20
13.00—13.40		70	57.30		Unclamped.
13.40		Clamped.			No secretion.
13.40—14.00		40	2.00.0	C_{20}	17
14.00—15.00		25	02.30		Clamped.
15.00—16.00		15	10.00	C_{10}	17
15.00—17.00		5	11.00—12.00		18
17.30		Unclamped.	12.00	C_{10}	15
17.30—18.00		20	12.30		Unclamped.
18.00—19.00		50	12.30—13.00		12
19.00—19.20		20	13.00—13.25		53
19.30—20.00		35	13.30—14.00		70
20.00—21.00		33	14.00—14.45		95
21.00—22.00		22	14.50—15.00		50
22.00—26.00		28	15.00—16.00		130
26.00—27.00		0	16.00—17.00		110
27.00		Clamped.	17.00—18.00		57
27.00—39.00		−27			Experiment stopped.

Experiment XXIV, Nov. 23. — Dog. 40 lbs. 4 c.c. 3 per cent morphine subcutaneous at 10 A.M. Perfusion of gland. Bled from carotid at 12.50 P.M. Blood whipped and put in perfusion apparatus. Bled as much as possible and then trachea clamped. At 1.08 began to admit blood into gland. Unable to read saliva at first, as it was below scale in cannula. No stimulation of either nerve.

Time. h. m. s. m. s.	Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
1.11.00–12.00	110	1.36.00–38.00		95
12.00–12.30	60	38.00–39.00		30
12.40–13.00	45	39.30–41.00		53
13.00–14.00	105	41.00–42.00		28
14.00–14.30	65	42.00–44.00		54
14.40–15.00	65	44.00	C_{10}	35
15.00–16.00	110	45.30–47.00		45
16.50–17.00	29	47.00	C_{20}	45
17.00–18.00	111	48.00–49.00		15
18.15–19.00	127	49.00–50.00		17
19.00–19.30	38	50.00–25.00		Clamped.
20.00–21.30	120	50.25–51.10		7
22.50–23.00	14	51.10–52.00		0
23.00–24.00	66	52.00–55.00		0
24.15	Clamped.	55.00		Unclamped.
24.15–24.30	13	55.00–56.00		0
24.30–24.45	1	56.00–57.00		4
24.45–25.00	1	57.00–58.00		5
25.00–31.00	7	58.00–59.00		9
31.00	Unclamped.	59.00–63.00		53
31.00–32.00	2	2.03.00	C_{20}	32
32.00–33.00	2	04.00–07.00		19
33.00–34.00	18	Secretes very slowly hereafter. Out-		
34.00–35.00	38	flow from vein almost nil. Stopped		
35.15–36.00	45	experiment.		

Experiment XXV. — Dog. Weight 25 lbs. 2 c.c. 3 per cent morphine sulphate at 9 A.M. Dog killed by bleeding at 10.45. Perfused gland.

Time. h. m. s. m. s.	Secretion in mm.	Time. h. m. s. m. s.		Secretion in mm.
10.57.00	Perfusion begins.	11.07.30–08.00		55
11.00.00 Adjust.	Duct kinked.	08.00		Clamped.
01.00–02.00	50	08.00–08.20		29
02.00–02.30	90	08.20–08.40		6
02.50–04.00	80	08.40–09.00		4
04.00–04.30	75	12.00	C_{10}	8
04.50–05.00	25	13.00	S_{20}	0
05.00–05.50	115	16.00		Unclamped.
06.00–07.00	145	16.00–17.30		5
07.00–07.15	25	17.30–18.00		7

Experiment XXV — (continued).

Time. h. m. s. m. s.	Secretion in mm.	Time. h. m. s. m. s.	Secretion in mm.
11.18.00—19.00	36	11.23.30—24.00	0
20.30—21.00	33		No more.
21.00—22.00	67	12.15.00	0
Inject atropine into arterial blood.			End of experiment.
22.00—22.30	17		

An inspection of the foregoing experiments shows that the spontaneous secretion which ensues on the readmission of the blood begins after a latent period of one to two minutes and reaches its maximum rate of flow in about five minutes. Thereafter it gradually decreases, persisting from fifteen minutes to half an hour, but ultimately coming to a stop. At its maximum rate of flow the gland of a large dog may secrete at the rate of nearly 2 c.c. per minute, though generally it is less than 0.76 of a cubic centimetre per minute. As a rule the saliva thus secreted appears to be thin. In one or two experiments the gland became œdematous; this was not, however, the rule. The secretion persists some time after the extreme dilation of the arteries has passed, when in fact the blood is issuing from the vein decidedly venous.

If during the height of the secretion the gland artery is again clamped the secretion is brought rapidly to a standstill. It practically ceases anywhere from 30 to 50 seconds after clamping. A back flow into the gland often takes place after the lapse of two or three minutes and persists slowly throughout the period of clamping. I have indicated such occurrences by the minus mark before the figures. This back flow as a rule is not large, but it may amount to 0.1 of a cubic centimetre. I am at a loss to account for it; the saliva was only a few centimetres above the level of the gland, a pressure altogether insufficient to cause back filtration, and I could discover no signs of leakage. It may have been due to a relaxation of the tissue of the gland. Occasionally a very slow flow of saliva persists from the duct through a good part of the time of clamping. This occurs generally in very large glands which have been secreting constantly and is probably but the passive forcing out by the distended gland of the stored-up saliva.

In the cat I have not found, in three experiments, a similar spontaneous secretion, but possibly I have not cut the blood supply off for the proper time. Attempts to bring this spontaneous secretion to pass in the dog by cutting off the blood supply to the

head by compression of the carotids, vertebrals and subclavians have also been fruitless, as, in the two dogs in which I tried this, the head was sufficiently supplied by some anastomosis through the vertebral column. The few cases in the foregoing experiments which have not shown a spontaneous secretion on readmitting the blood are due, I am convinced, either to the kinking of the gland artery or to a failure to cut off the blood supply.

How shall this spontaneous and long continued rapid secretion be explained? Two possibilities naturally suggest themselves. It may be due to the action of the nerve cells lying in the hilus of the gland, or it may be due to the action of the gland cells. The first possibility appears to me to be altogether unlikely. So far as I know, there is no analogy elsewhere of mammalian nerve cells stirred up to continuous activity by the action of oxygen after being deprived of blood for from fifteen minutes to half an hour. On the contrary, nerve cells are as a rule placed decidedly *hors de combat* by such treatment. Furthermore we have a strictly analogous secretion in the sweat glands, in which no nerve cells are known to exist, and the probably analogous case of the spontaneous sweat secretion in the horse following division of the sympathetic. I believe therefore that we may safely abandon this hypothesis.

This conclusion is rendered still more certain by Levy's observations. Levy found that the sweat glands secreted spontaneously after being deprived of blood for a *minimum* time of three hours and twenty-five minutes. Even if nerve cells exist in the sweat glands — and their existence there has never been shown — it can hardly be believed that four hours after deprivation of oxygen they are capable of causing this secretion. Stimulation of the nerves at such a time is without effect.

The second possibility, *i. e.*, that the secretion may be due to the action of the gland cells themselves appears, more probable. Hoppe-Seyler long ago pointed out from chemical evidence that when oxygen is taken away from protoplasm, hydrolytic decompositions take place in the protoplasm leading generally to the formation of acid. This fact has been abundantly confirmed microscopically by Loeb,[1] Budgett,[2] Zoethout,[3] Kühne and others,[4] so that any one

[1] LOEB: Archiv für die gesammte Physiologie, 1895, lxii, p 249.
[2] BUDGETT: This journal, 1898, i, p. 210.
[3] ZOETHOUT: This journal, 1899, ii, p. 220.
[4] LOEB und HARDESTY: Archiv für die gesammte Physiologie, 1895, lxi, p. 583.

who will place infusoria, ova, or other cells under the microscope and deprive them of oxygen may see that they liquefy, absorb water, and burst. There is no manner of doubt, then, unless gland cell protoplasm differs from all other, that it must follow the same rule. As a matter of fact this behavior of protoplasm is particularly well illustrated in the normal life of the gland cell. During a period of rest of the gland the blood supply is cut down so that the gland is decidedly anæmic. At this time it may be seen by a study of sections that the protoplasm of the cell does break down, or is transformed, into the so-called secretory products, and the cells greatly increase in size. This last fact is undoubtedly a consequence of the first, the decomposition increasing the osmotic pressure of the cell contents and bringing about an imbibition of water. Last summer at Woods Holl I observed that eggs of the sea-urchin Arbacia liquefy with far greater ease if exposed to oxygen intermittently during periods of deprivation, than if kept in an atmosphere of hydrogen. There is very good ground, hence, for the belief that gland cells deprived of oxygen do undergo hydrolysis, or decomposition, leading to liquefaction, that their osmotic pressure is thereby greatly increased, and that the final processes of decomposition are greatly facilitated by the renewal of oxygen. The facts that this spontaneous secretion is accompanied by vasodilation long continued, the cells being literally overwhelmed with oxygen, and that the secretion is closely dependent on the blood flow, stopping with remarkable quickness when the blood flow is cut off, strongly bear out this hypothesis. It may be recalled in this connection that the chorda can cause a secretion at least five minutes after clamping the artery, so that if we were dealing with a nerve cell activity, the gland might be expected to continue to secrete for a much longer period.

For these reasons, I believe that this spontaneous secretion must be due to the action of the gland cells and not in any way whatsoever to the action of any hypothetical secretory nerves.

If this secretion is thus due, as I believe, to the gland cells, the action of atropine upon it is of the highest interest. Atropine stops the secretion suddenly and permanently. It must have acted hence on the gland cells themselves since no paralysis of the secretory nerve end would be efficacious. If this is so the secretory nerve theory loses one of its strongest supports.

It may seem to some that the fact that atropine checks this secretion

is clear evidence that the secretion must be due to secretory nerves. That some should so contend would not be unnatural, so firmly rooted has the idea become that the drug is an infallible agent for the detection of these endings. All who would so believe should remember that the secretory nerves are in a large measure assumed to exist in order to explain the action of the drug, and hence the drug can no longer be considered as evidence of the existence of such nerves, so soon as any other possible explanation of its action is forthcoming. In this case such another explanation has been offered, not as a remote possibility, but, I believe, as a decided probability. I hope in the near future to come back to the anti-secretory action of atropine more at length.

SUMMARY.

1. If the blood supply is cut off from the submaxillary gland of the dog for 12–25 minutes, on readmission of the blood the gland secretes rapidly and continuously for several minutes. This secretion is accompanied by a marked vasodilation and is probably due to the increased osmotic pressure of the cells of the gland following the deprivation of oxygen and its readmission.

2. This secretion is closely dependent on the blood flow. It generally ceases within a minute after clamping the artery.

3. The secretion is paralyzed by atropine. The drug atropine must hence act directly on the gland cells and its value as a witness for secretory nerves is seriously impaired.
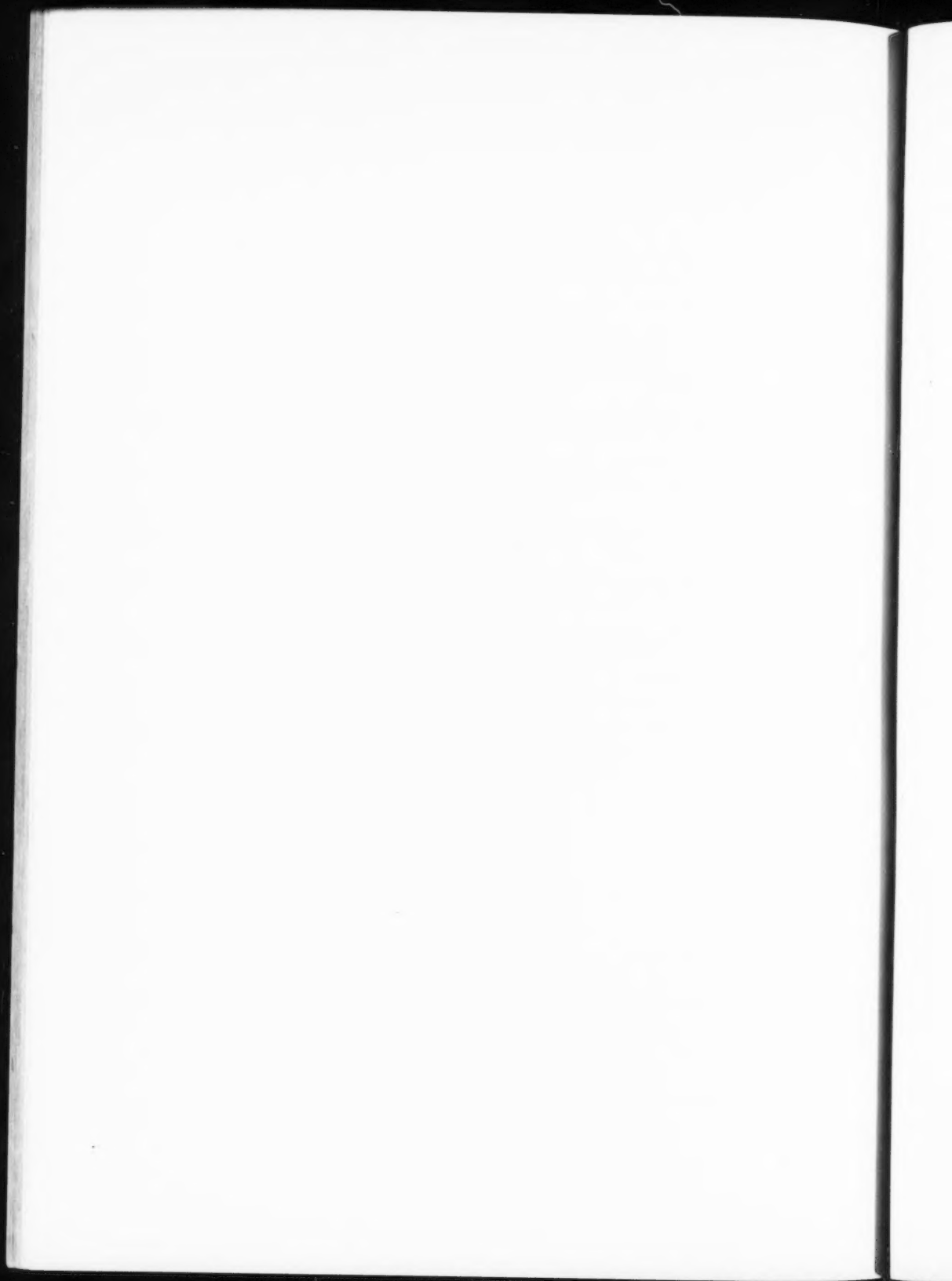
PROCEEDINGS OF THE AMERICAN PHYSIO-
LOGICAL SOCIETY.

FIFTH SPECIAL MEETING.

Washington, D. C., May 1 and 2, 1900.
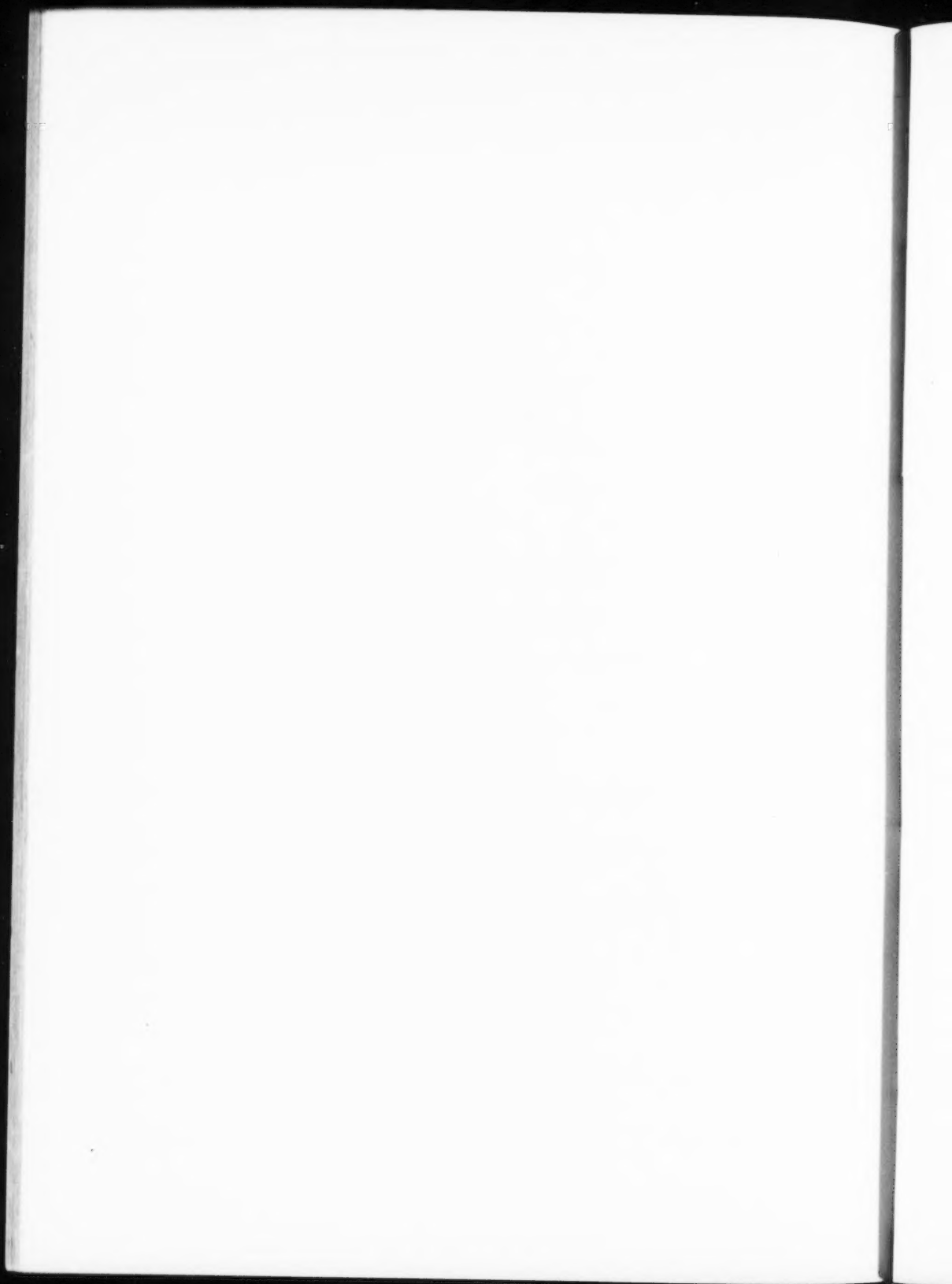
PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

A CONVENIENT FORM OF PRESSURE-BOTTLE.

By W. P. LOMBARD (FOR MR. A. E. GUENTHER).

IT is not always desirable to conduct a blood-pressure experiment at a part of a laboratory where a pressure-bottle can be suspended, and the method of filling the manometer, etc., by means of an ordinary syringe is often inconvenient. The pressure needed to drive the anticoagulation fluid into the manometer, and the tubes connecting with the artery, can be obtained by compressing the air in the bottle containing the fluid by means of an ordinary bicycle-pump. A Wolff bottle with three openings, *a*, *b*, *c*, closed by rubber stoppers, can be used to advantage. Through *a* a glass tube of small bore passes to the bottom of the bottle, and serves to conduct the fluid to the tube connecting with the manometer; a tube communicating with the air at the top of the bottle is inserted through *b*, and the third stopper carries the tube having the valve of the pump. Where they leave the bottle tubes, *a* and *b* must connect with rubber tubes closed with clamps. Enough fluid may be put into the bottle to last for several experiments, and then the stoppers may be fastened in so as to be air tight. If the clamps on *a* and *b* are closed, a few strokes of the pump will bring up the air pressure in the bottle sufficiently to drive out all the fluid needed to fill the manometer and connecting tubes (one must avoid giving too great a pressure, as it is possible to burst an ordinary bottle). When the manometer is to be filled, at the same time that clamp *a* is opened, it is best to keep the rubber tube connected with it partly closed with the fingers, the better to control the rate of exit of the fluid. At any time when it is desired, the pressure in the bottle can be relieved by opening the clamp at *b*.

EARTH–CURRENTS OBSERVED AT THE PHYSIOLOGICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN.

By WARREN P. LOMBARD.

ATTENTION was called to the existence of these currents during certain experiments on nerve-muscle preparations, in which unexpected twitchings were observed. The behavior of the preparations suggested that they were charged with static electricity. A new electric light plant had just been installed, and it occurred to us that there might be a spread of current from this source. When a lightning-rod on the building and a gas-pipe were connected with a Wesson's milammetre, irregular movements of the index were observed, showing that the iron work of the laboratory was the seat of irregular fluctuations of electrical potential. When the nerve-muscle preparation was touched with the wires connected with the gas-pipe or water-pipe and the lightning-rod, it received a shock which caused a vigorous contraction.

It was soon found that the electric plant of the University was not responsible for the disturbance, but that it was due to a spread of current from electric car lines. There are two lines in our vicinity, — the one makes a loop about the University, the other runs to one side of the University, and about half a mile from it at the nearest point, and connects Ann Arbor with the town of Ypsilanti about seven miles away. Examination of the time of movement of the cars on these two lines showed us that the currents from the near line were very weak, and that all the large electrical variations observed came from the more distant line. By recording the movements of the index of the milammetre, and below this the time in minutes, on a blackened drum, we obtained a record of the starting and stopping of the cars on the Ypsilanti line from the time that they reached a point opposite the University, all the way to Ypsilanti; and the currents were found to be the stronger the further the cars were from the University. The movements of the cars from the point on the line opposite to the University to the business part of the city of Ann Arbor gave us no currents. This was explained by the fact that the power-house for the line was situated on the opposite side of the city from the University, and so the laboratory was out of the course of the earth-currents returning from that part of the line

to the power-house. During the past year the power-house has been moved from Ann Arbor to Ypsilanti, and we cannot now detect any earth-currents such as we observed under the old arrangement. Our galvanometers, which had formerly exhibited annoying fluctuations of the O point, behave very much better, and we no longer see the peculiar twitchings of the nerve-muscle preparations which were the cause of our investigation.

Our study of this subject has convinced us that it is the position of the electric power-plant, rather than the nearness of an electric line, which decides whether a laboratory shall be invaded by street car currents. A laboratory half a mile from an electric line, if in the path of the earth-currents, will be more influenced by them than another which may be situated close to the car track. Probably the presence of water conductors, streams, etc., would have a marked influence on the direction which the earth-currents would take.

ON THE DECREASE IN THE PROPORTION OF WATER IN THE CENTRAL NERVOUS SYSTEM OF THE GROWING WHITE RAT.

By HENRY H. DONALDSON.

THESE observations have been made in conjunction with Mr. H. J. Polkey. From white rats of different ages the brain and spinal cord were removed separately, and the proportion of water in them determined by drying at about $97°$ C. A study of the results shows that at birth the brain contains about 88 per cent of water and the cord 85 per cent; whereas, in old age, the brain contains about $77\frac{1}{2}$ per cent and the cord 70 per cent. A difference in the percentage of water exists between the brain and cord at birth, and increases steadily throughout life.

In both brain and cord, the curve representing the diminution in the percentage of water can be divided into three parts. The first part covers the first eight to ten days after birth. During this time, the diminution in the percentage of water is slow. The second part comprises the next forty days of life. During this period, the loss is very rapid. The third part is from the end of the second period to

the termination of life, during which there is a very slow but steady diminution in the percentage of water.

The principal change in the central nervous system, correlated with the proportional loss of water, is the formation of medullary sheaths. This formation is comparatively slow during the first period, very rapid during the second, at the end of which the brain has finished its period of rapid growth; and slow during the third period, when medullary substance is added slowly but steadily until life ends.

After medullation the excess of medullary substance in the cord, as compared with the brain, accounts for the difference in the percentage of water. At birth there are no medullary sheaths, and the greater percentage of water in the brain is explained by the greater proportion of cell-bodies as contrasted with axones in the brain, the cell-bodies possessing the higher percentage of water.

The test of this correlation will be the determination of the amount of medullary substance at different ages, and experiments to this end are at present in progress.

THE FUNCTIONAL SIGNIFICANCE OF THE SIZE AND SHAPE OF THE NEURONE.

By HENRY H. DONALDSON.

THESE observations were made upon growing nerve cells in the white rat, as they appear between birth and maturity. In the growing spinal ganglion of the lumbar nerves, the increase in volume of the largest ganglion cell-bodies was shown to be very closely correlated with the increase in the area of a cross section of the nerve fibre growing out of these cell-bodies. The examination of the fibres was made on the peripheral side of the ganglion.

Further study of the cross section of the nerve fibre showed that the area of the axis cylinder was almost exactly equal to the area of the surrounding medullary sheath. This held true from the time that the medullary sheath was completely formed on the largest fibres of the sciatic nerve.

In the new-born rat, the sciatic fibres are totally unmedullated, and there is a short preliminary period in which not only the axis cylinder increases, but the medullary sheath is added, and so rapid

is this first formation of the sheath that in a few days it attains the relation just mentioned.

This equality in the areas of the sheath and axis persists through the entire growing period of the white rat. There are thus two phases in the growth of the medullary sheath represented, first, by its rapid appearance, and second, by its subsequent slow enlargement. It was shown from the data of comparative anatomy that the *length* of the nerve fibre from a given cell was not correlated with the volume of the cell-body, and was apparently a matter of small significance; the increased length of the fibre not putting a direct nutritional tax on the cell-body itself.

In this connection, the observations of Dr. Elizabeth Dunn were also presented. These showed that the current dictum that the nerve fibres of larger calibre had the longer course did not hold true in the case of the nerve fibres supplying the thigh of the frog; here it could be demonstrated that the average diameter of the fibres innervating the thigh was greater than the average diameter of the fibres passing beyond the knee to innervate the remainder of the leg. Moreover, the branches going to the thigh contained the very largest fibres that are found in the sciatic nerve.

This statement applies to the several physiological classes of fibres taken all together, and does not distinguish between the afferent and efferent axones.

Finally, an interpretation of the calibre of the nerve fibre was attempted, and it was pointed out that fibres of large calibre tended to have an extensive terminal distribution. Where the nerve elements were few in number as compared with the mass of skin or muscle to be supplied, this indicated a coarse innervation. If, however, the number of nerve elements was large, a very fine degree of innervation might result, as in the case of the extrinsic muscles of the eye.

ON THE SULPHOCYANIDE-CONTENT OF HUMAN SALIVA.

By LAFAYETTE B. MENDEL AND E. C. SCHNEIDER.

A BRIEF résumé of a large number of observations on the variations in the sulphocyanide-content of the saliva of different individuals was presented. The chief points of interest are: The almost

constant presence of KSCN in the saliva of man, and the marked difference between the saliva of smokers and non-smokers as regards the content of KSCN. The observations confirm the recent statements of Krüger; the saliva of non-smokers showed an average of only 0.0029 per cent KSCN, whereas that of smokers indicated 0.0134 per cent.

The saliva from the Stenonian duct is uniformly richer in KSCN than that collected from Wharton's duct. Thus far it has not been possible to correlate these differences with other variations in composition. The saliva from the individual glands also shows the characteristic intensity of KSCN reaction associated with smoking. An outline of the methods employed and additional observations will be presented later.

THE ACTION OF CERTAIN TOXIC PRODUCTS OF THE TYPHOID BACILLUS ON THE HEART.

By GEORGE T. KEMP and MISS S. L. DEWEY.
(Preliminary communication.)

1. THE liquid media ordinarily used for the cultivation of bacteria, containing, as they do, extractives, proteoses, and peptone, are unfit for irrigation of the heart, as they produce decided effects of their own and mask the effect of any bacterial products formed in them.

2. To prepare a fluid free from these objections, proceed as follows: Precipitate egg albumen with $(NH_4)_2SO_4$, remove the salt thoroughly by dialysis, dissolve the precipitate in a weak solution of NaOH (the strength of the NaOH was about $\frac{N}{10}$). Take about one egg-white to 100 c.c. of the solution. The alkaline solution of albumen is next treated with HCl to a point just short of neutralization, when the albumen is barely held in solution. This is now added to Ringer's solution, so that:

<div align="center">Albumen solution : Ringer's solution :: 1 : 10.</div>

3. A fluid prepared as above has an action on the heart practically indistinguishable from that of Ringer's solution. It forms an excellent medium for the culture of the typhoid bacillus.

4. To test the action of toxic products of the bacillus, the cultures were filtered through a Berkefeld filter, and the bacteria-free liquid used (unfiltered cultures gave practically the same results).

5. This liquid was added in varying percentages to Ringer's solution, and its action on the heart recorded.

6. Terrapin hearts were used, and the species of the terrapins noted all gave the same results.

7. Ringer's solution, containing 12.5 per cent of the culture, gave a marked effect. The effects from a 50 per cent solution were always prompt, strong, and decided.

8. The heart-beat was gradually weakened without being slowed, and the heart was finally stopped in systole (the vagi were cut).

9. The rapidity of the effect depends upon the strength of the solution.

10. Hearts could be revived by flushing with Ringer's solution, if the poison had not acted too long. It usually took about four times as long for the heart to regain its normal beat as it had taken to fall from the normal to stoppage. After strong solutions of the poison, the heart could not be revived.

11. To determine to what class of poisons the active substances belong, proceed as follows: Filter the culture. Precipitate with alcohol about 98 per cent or over. Wash, precipitate with alcohol. Dry in desiccator. Dissolve in Ringer's solution. The dissolved precipitate gives the same kind of effect on the heart as the culture, but is somewhat less powerful. It must be an albumin or a globulin.

12. The fact that the precipitate described in 11 gave a less powerful action than the culture, suggested the possible existence of some other active substance whose effect might be opposite to that of the one described.

13. Such a substance was found in the alcoholic filtrate in 11.

14. The filtrate, when evaporated to dryness and dissolved in Ringer's solution, gave the following result: Its presence caused a marked increase in the strength of the heart-beat without affecting the rate.

THE ACTION OF PHLORHIZIN ON MUSCLE.

By FREDERIC S. LEE and C. C. HARROLD.

In investigating the causes of muscle fatigue the action of phlorhizin on muscle has been studied. One gram of phlorhizin dissolved in sodium carbonate was injected three times a day into fasting cats, and the administration of the drug was continued for from two to

over four days.   The animals were then killed and the course of
fatigue in the tibialis anticus was studied.   Instead of giving 800 to
1000 contractions, of which the normal muscle is capable, the phlo-
rhizinized muscle gives 200 to 400.   The curves of contraction of the
latter clearly resemble the later contraction curves of the normal
muscle when undergoing fatigue, being low in height and the phase
of relaxation being somewhat prolonged.   The phlorhizinized muscle
hence is comparable to the normal muscle in the late stages of
fatigue.

Is this result due to a specific action of the drug on the protoplasm
of the muscle cells, or to the loss of carbohydrate ?   Irrigation of
the muscle with phlorhizin dissolved in sodium carbonate diminishes
the number of contractions, but irrigation with sodium carbonate
alone has the same effect.   Hence it is probable that phlorhizin has
no specific action apart from its influence on the organism which
results in the removal of carbohydrates.   This conclusion has been
tested in another way.   Animals were given phlorhizin for four days
in the usual way, and then 50 grams of dextrose were administered by
the stomach.   Eight hours afterward the animals were killed, and the
muscle fatigue was studied.   Such muscles gave 650 contractions,
the first 100 of which were quite normal, the later ones showing the
lengthened relaxation.   The dextrose had counteracted the effect of
the phlorhizin and largely restored the muscle.

The provisional conclusion is strongly suggested that the loss of
carbohydrate is an important factor in the early phases of muscle
fatigue.   Moreover, the administration of dextrose would seem to
bring the muscle out of its fatigued condition.   No conclusive
chemical tests have yet been made, but they will be performed later.
It will be necessary to test the phlorhizined muscle, both as to the
actual loss of carbohydrate and the possible accumulation of products
of proteid decomposition.

Incidentally some observations on rigor have been made.   A well
phlorhizinized muscle begins to go into rigor within five minutes after
death, and rigor may be complete within twenty to thirty minutes.
This accords well with Miss Latimer's experimental results.[1]   Irriga-
tion of a muscle with dextrose does not, however, destroy the irrita-
bility of the muscle to direct stimulation.   On the contrary, a muscle
so irrigated is capable of giving one thousand contractions, fully as
many as, or more than, a normal muscle without dextrose.

[1] See this journal, 1898, ii, p. 29.

THE INFLUENCE OF PHLORHIZIN DIABETES ON LACTATION.

By GRAHAM LUSK.

If a well fed milch goat be made to fast two days and phlorhizin be administered three times daily during the two days, the milk flow stops entirely. If the phlorhizin be then discontinued and the goat fed, the function of lactation returns, although in diminished intensity. If the phlorhizin administration be continued four or five days in a fasting goat, the power to produce milk does not return on the simultaneous cessation of the diabetes and the fast. It is probable that the stoppage of the milk depends on the diabetes and not on a specific action of phlorhizin on the gland. In the sugar elimination, the fasting goat resembles the rabbit, since in the urine is found the ratio :

Dextrose : Nitrogen :: 2.8 : 1.

THE CHEMISTRY OF PARANUCLEO COMPOUNDS.

By P. A. LEVENE AND C. L. ALSBERG.

THE object of the work was to investigate the relation of paranucleic acids to the true nucleic acids. The substances investigated were ovovitellin and ichtulin of the codfish egg. The following results were obtained:

1. The ichtulin of the codfish differs from that described by Walter in its percentage composition and in the fact that it does not contain a carbohydrate in its molecule.

2. Both ovovitellin and ichtulin yield, on treatment with alkalies, substances akin in some of their properties to true nucleic acids.

3. These substances differ from nucleic acids not only in the absence of purin bases in their molecule, but also in the fact that they contain in their molecule a proteid.

4. The later proteid does not resemble the protamines, as can be concluded from the amount of " hexon " bases which it yields.

5. The iron enters the molecule of the paranucleins in a combination probably similar to that of the ethereal acids.

CONTRIBUTIONS TO THE CHEMISTRY OF THE LYMPHATIC GLANDS.

By LAFAYETTE B. MENDEL AND R. NAKASEKO.

MENDEL and Jackson have failed to observe any diminution in uric acid production in the dog and cat after splenectomy, although the formation of uric acid by the action of spleen pulp under appropriate conditions has been demonstrated. In view of a possible compensatory development and function of the lymphatic glands after removal of the spleen, the Horbaczewski-Spitzer experiments were repeated with lymphatic tissue. Lymphatic glands found imbedded in the pancreas of the sheep and calf, as well as those taken from the submaxillary region of the ox, were used. At most, only traces of uric acid were obtained by treatment of 100 to 300 grams of tissue. Xanthin bases were found in apparently larger quantity. The glands are rich in nucleic acid, the study of which is being continued.

APPARATUS FOR RECORDING CONTRACTIONS, BY LOCALIZED UNIPOLAR EXCITATION OF THE NERVE, OF AN ISOLATED NERVE–MUSCLE PREPARATION.

By WARREN P. LOMBARD.

PROFESSOR W. KÜHNE, of Heidelberg, employs unipolar excitation most successfully in many forms of experiment in which a strict localization of excitation is required, *e.g.*, to demonstrate isolated conduction of muscle fibres; the transmission of the nerve impulse in both directions along the nerve; the function of different parts of the brain cortex of the frog, etc. He places the muscle, nerve-muscle, or the whole frog on an isolated metal plate, connects this with one pole of the secondary coil of an induction apparatus (the other pole being connected with a gas-pipe and so with the earth), puts an automatic interrupter in the primary circuit, and then touches the point to be excited with a fine needle held in the hand. When the primary circuit is closed, and the automatic interrupter is in motion, the electric potential of the preparation undergoes rapid

alterations, but the preparation is not excited unless it is touched with the needle; a sudden change of electric potential does not excite; the sudden flow of a dense electric current is essential to the excitation process, and this occurs when some point on the nerve is touched by the needle held in the hand of the observer.

The apparatus which I wish to demonstrate, involves the principle employed by Kühne, and permits the recording of the contractions of a muscle when it is indirectly stimulated by unipolar excitation of its nerve. I have substituted for the metal plate employed by Kühne, a sheet of thin gold foil, which is a good conductor and has the advantage that it is very flexible and can be kept in contact with the surface of a large part of the muscle without impeding its movements. A strip of the foil is spread over and is allowed to hang down from one end of a block of vulcanite which is fastened by a glass rod and suitable clamp to a stand. The muscle is suspended from a vulcanite clamp fastened to the block of vulcanite, at the end from which the gold foil is hanging. When the muscle is in place, the gold foil is wrapped around it and the nerve is laid on the foil on the block so that it is everywhere in contact with the gold. The muscle can now be connected with the recording lever by a thread which has been boiled in paraffine, the paraffine being used to prevent the thread from taking up moisture, care must be taken in moistening the muscle that the fluid does not run down the thread. If the insulation fails to be complete at any point, there will be a flow of current there and consequent excitation.

If the gold foil be connected with the secondary coil of an induction apparatus, arranged as described above, and the interrupter be started, the nerve can be locally excited by touching it with a piece of gold wire held in the hand, and the resulting contraction of the muscle recorded. It is more convenient to connect the piece of gold wire with a good metal conductor, such as a sheet of tin foil. The conductor must have a large surface, eight or ten square feet will suffice, and may be either suspended or rolled up with layers of paraffine paper separating the succeeding turns. It is not necessary that the conductor should be in connection with the earth, and a circuit established through this with the secondary coil; there will be a flow of current through the part of the nerve touched by the gold wire when the conductor is being charged and discharged, even when it is wholly insulated. That the excitation is strictly localized can be readily proved by tying a ligature about the

nerve, and applying the gold wire to the nerve close to the ligature, first below and later above the ligated point; in the first case a contraction of the muscle will be recorded, in the second case there will be no contraction.

Both making and breaking induction shocks can be used to excite, but the former is effective only when the primary current is very strong.

THE USE OF ALKALINE SOLUTIONS IN SURGICAL SHOCK.

By W. H. HOWELL.

THE paper called attention to the very striking individual differences shown by healthy animals to serious operations, some maintaining a good blood-pressure and normal heart-rate, while others quickly fall into a condition of shock exhibiting a very low blood-pressure and a rapid feeble heart-beat. It was shown also that these two important vascular symptoms of shock are often dissociated. In operations upon the brain especially, a condition of cardiac shock often ensues, the heart-rate being increased one hundred per cent or more, and the beat becoming feeble, while the blood-pressure remains within normal limits. In most of the experiments reported, shock was produced by operations upon the cerebrum, without marked hemorrhage, and during this condition alkaline solutions (Na_2CO_3, 5 per cent or ½ per cent) were injected directly into the veins or into the rectum. It was found that in conditions of moderate shock in which the blood-pressure remained as high as 60 to 70 mm. of mercury, the alkaline solutions brought the pressure back to permanently normal limits, 100 mm. or more, and caused a marked increase in the force of the heart-beat. In conditions of profound shock in which the blood-pressure had fallen to 20 to 30 mm. of mercury, the alkaline solutions restored the pressure to about 60 to 70 mm. and brought back a strong heart-beat. The effects obtained under these circumstances were relatively permanent, lasting for one or more hours, and it was suggested that repeated injections at certain intervals might result in a permanent recovery of normal vascular tone. Attention was called to the fact that when the alkaline solutions are injected directly into the veins,

care must be taken not to use an excessive amount, not more than sufficient to increase the total alkalinity of the blood by 0.1 to 0.2 per cent. Rectal infusions of 0.5 per cent Na_2CO_3 in normal saline were recommended as a safer procedure. Experiments made upon the serum obtained from the blood of an animal in a condition of shock, and injected into the veins of a normal animal, indicated that shock blood contains no poisonous substances.

PLETHYSMOGRAPHIC EXPERIMENTS UPON HYPNOTIC SLEEP. By E. C. WALDEN.
This Journal, 1900, iv, p. 124.

NOTE ON MOSSO'S SPHYGMOMANOMETER. By E. C. WALDEN.
This Journal, 1900, iv, p. 128.

A CHEAP SUPPORT FOR HAND DRUMS. By W. P. LOMBARD.

ARTIFICIAL CIRCULATION IN THE ISOLATED KIDNEY. By FRANZ PFAFF and M. P. O. VEJUX-TYRODE.

IS THE ACTIVITY OF THE DIGESTIVE FERMENTS INHIBITED OR STOPPED IN A SATURATED AQUEOUS SOLUTION OF CHLORETONE? By T. B. ALDRICH.
Read by title.

A NEW RHEOCHORD. By E. T. REICHERT.
Read by title.